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Gallo et al.

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(54) **MOLECULAR CLONING OF HIV-1 FROM IMMORTALIZED CELL LINES**

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Related U.S. Application Data

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C12Q 1/68 (2006.01)
C12Q 1/70 (2006.01)

(52) **U.S. Cl.**
CPC **C12Q 1/703** (2013.01); **C12Q 1/6834** (2013.01); **C12Q 1/6876** (2013.01)

(58) **Field of Classification Search**
USPC 435/235, 252.3, 240.2, 172.3, 69.1, 435/236, 320.1, 91, 91.1, 91.32, 91.4; 536/27
See application file for complete search history.

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(57) **ABSTRACT**

Disclosed is the molecular cloning of HTLV-III, the adult leukemia and acquired immune deficiency syndrome (AIDS) virus. Clone BH10 contains a 9.0 Kb viral insert constituting the entire HTLV-III genome. Clones BH8 and BH5 contain viral inserts of 5.5 Kb and 3.5 Kb, respectively. These clones are suitable for the development of diagnostic and therapeutic measures for AIDS, as well as use as probes for the detection of AIDS. By scientific convention, HTLV-III, referred to herein also as HIV, has been renamed as HIV-1.

161 Claims, 16 Drawing Sheets

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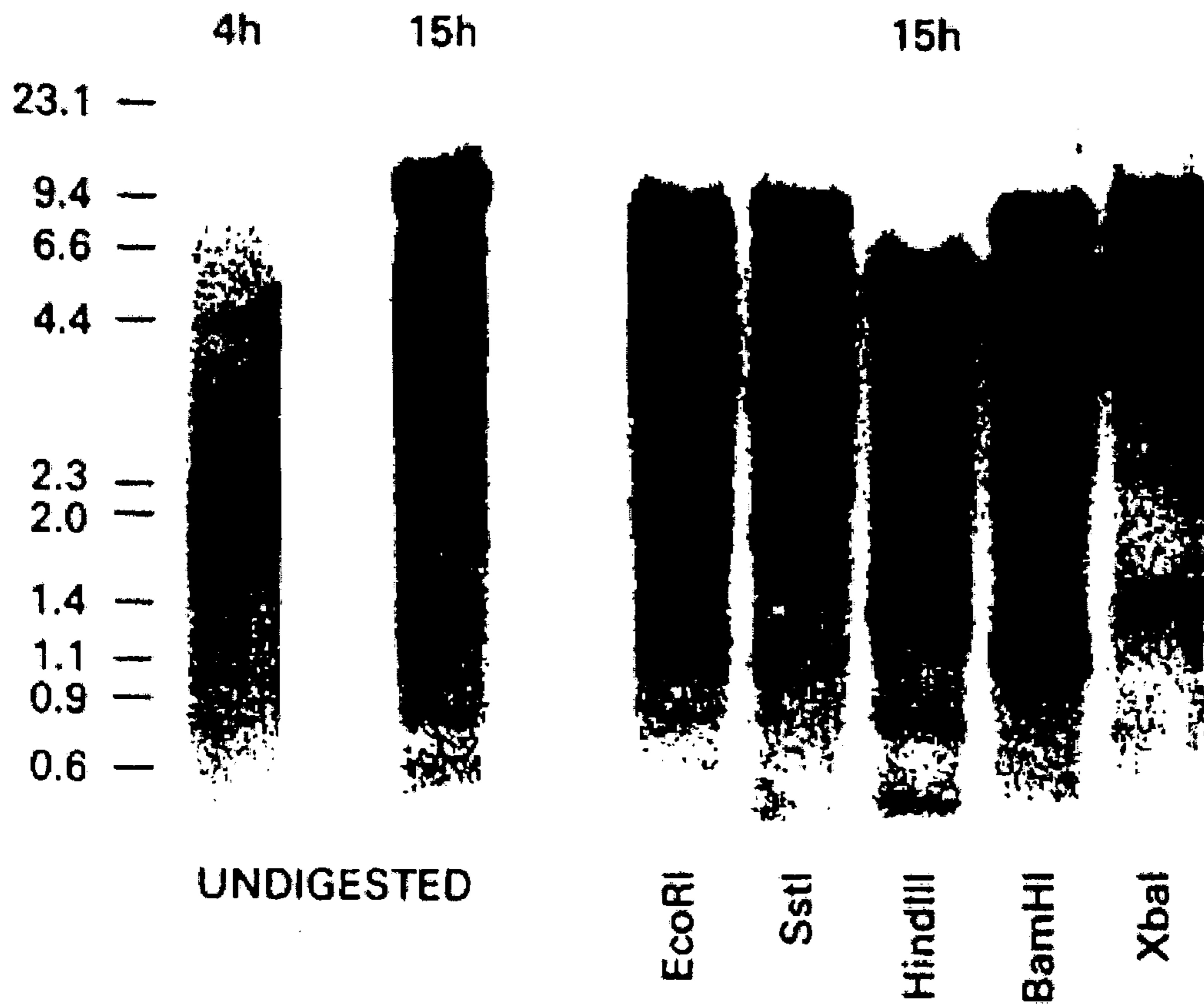


FIG. 1

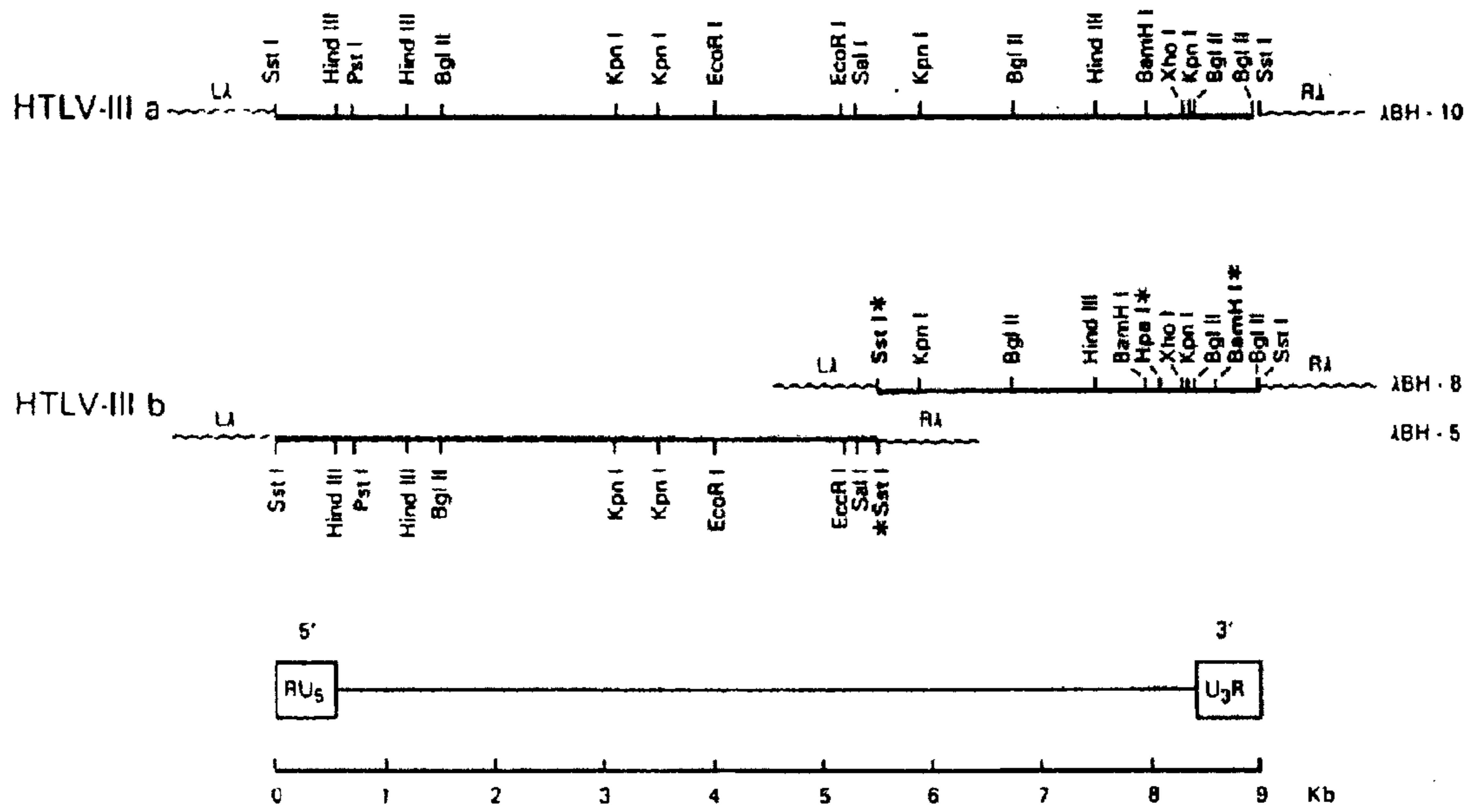


FIG. 2

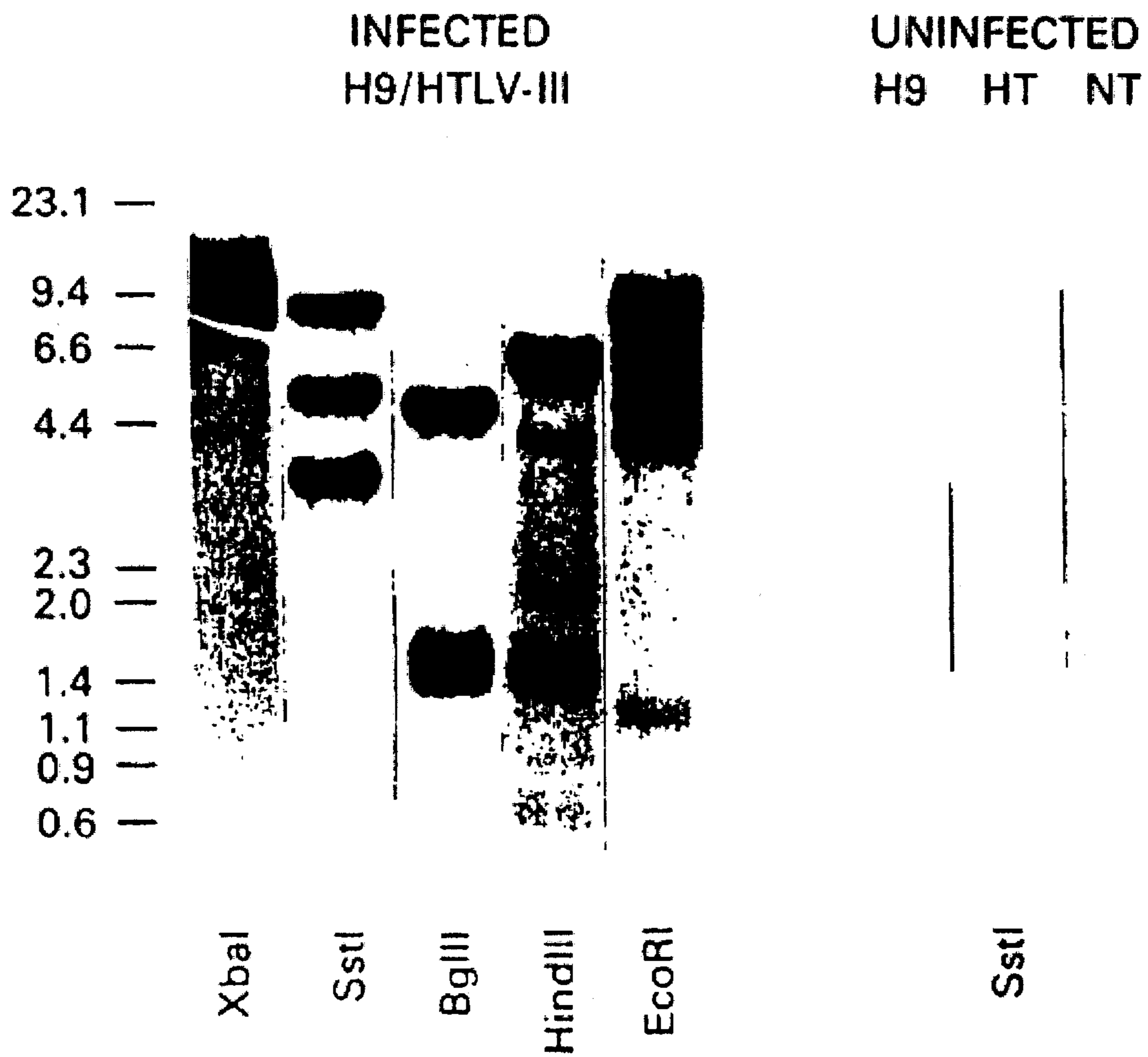


FIG. 3

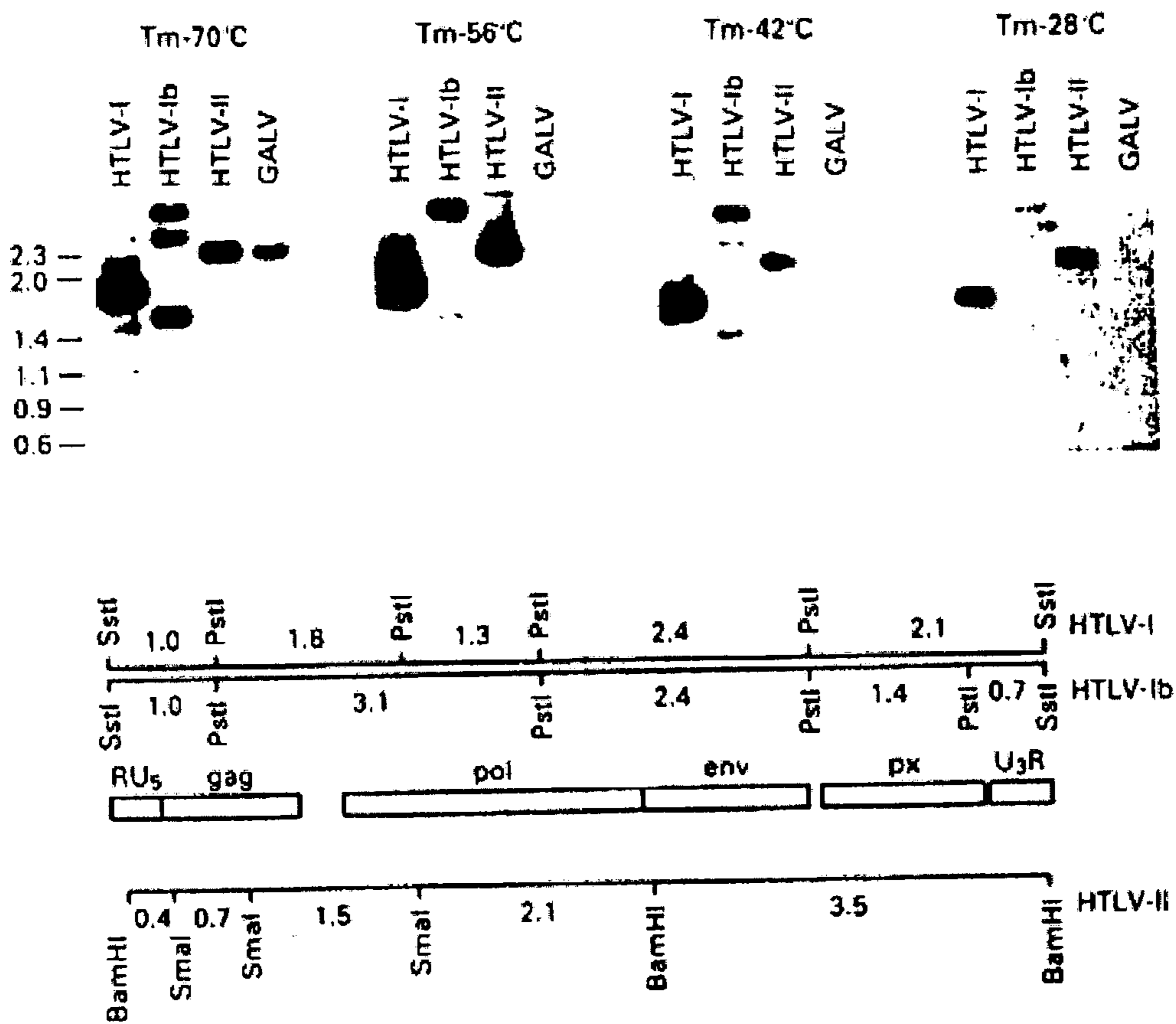


FIG. 4

GAGCTCTCTCGACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGGCGAGGGGCGG 60
CGACTGGTGAGTACGCCAAAAATTTGACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGC 120
GAGAGCGTCAGTATTAAGCGGGGAGAATTAGATCGATGGGAAAAAATTCGGTTAAGGCC 180
AGGGGGAAAGAAAAATATAAATTAAAACATATAGTATGGGCAAGCAGGGAGCTAGAACG 240
ATTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAAATACTGGGACA 300
GCTACAACCATCCCCTCAGACAGGATCAGAAGAACTTAGATCATTATATAATACAGTAGC 360
AACCTCTATTGTGTGCATCAAAGGATAGAGATAAAAGACACCAAGGAAGCTTTAGACAA 420
GATAGAGGAAGAGCAAAACAAAAGTAAGAAAAAAGCACAGCAAGCAGCAGCTGACACAGG 480
ACACAGCAGTCAGGTCAGCCAAAATTACCCTATAGTGCAGAACATCCAGGGGCAAATGGT 540
ACATCAGGCCATATCACCTAGAACTTTAAATGCATGGGTAAAAGTAGTAGAAGAGAAGGC 600
TTTCAGCCCAGAAGTAATACCCATGTTTTTCAGCATTATCAGAAGGAGCCACCCACAAGA 660
TTTAAACACCATGCTAAACACAGTGGGGGGACATCAAGCAGCCATGCAAATGTTAAAAGA 720
GACCATCAATGAGGAAGCTGCAGAATGGGATAGAGTGCATCCAGTGCATGCAGGGCCTAT 780
CGCACCAGGCCAGATGAGAGAACCAAGGGGAAGTGACATAGCAGGAECTACTAGTACCCT 840
TCAGGAACAAATAGGATGGATGACAAATAATCCACCTATCCAGTAGGAGAAATTTATAA 900
AAGATGGATAATCCTGGGATTAATAAAATAGTAAGGATGTATAGTCCTACCAGCATTCT 960
GGACATAAGACAAGGACCAAAGGAACCCTTTAGAGACTATGTAGACCGGTTCTATAAAAC 1020
TCTAAGAGCCGAGCAAGCTTCACAGGAAGTAAAAAATTGGATGACAGAAACCTTGTTGGT 1080
CCAAAATGCGAACCCAGATTGTAAGACTATTTTTAAAAGCATTGGGACCAGCAGCTACTCT 1140
AGAAGAAATGATGACAGCATGTCAGGGAGTGGGAGGACCCGGCCATAAAGCAAGAGTTTT 1200
GGCTGAAGCAATGAGCCAAGTAACAAATTCAACTACCATAATGATGCAAAGAGGCAATTT 1260
TAGGAACCAAAGAAAGATTGTTAAGTGTTC AATTGTGGCAAAGAAGGGCACATAGCAAG 1320
AAATTGCAAGGCCCTAGAAAAAGAGGCTGTTGGAAATGTGGAAAGGAAGGACACCAAAT 1380
GAAAGATTGTACTGAGAGACAGGCTAATTTTTTAGGGAAGATCTGGCCTTCCTACAAGGG 1440
AAGGCCAGGGAATTTCTTCAGAGCAGACCAGAGCCAACAGCCCCACCATTTCTTCAGAG 1500
CAGACCAGAGCCAACAGCCCCACCAGAAGAGAGCTTCAGGTCTGGGGTAGAGACAACAAC 1560
TCCCTCTCAGAAGCAGGAGCCGATAGACAAGGAACTGTATCCTTTAACTTCCTCAGATC 1620
ACTCTTTGGCAACGACCCCTCGTCACAATAAAGATAGGGGGGCAACTAAAGGAAGCTCTA 1680
TTAGATACAGGAGCAGATGATACAGTATTAGAAGAAATGAGTTTGCCAGGAAGATGGAAA 1740
CCAAAATGATAGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTC 1800
ATAGAAATCTGTGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAAC 1860
ATAATTGGAAGAAATCTGTTGACTCAGATTGGTTGCACTTTAAATTTTCCATTAGTCCT 1920
ATTGAAACTGTACCAGTAAAATTAAGCCAGGAATGGATGGCCCAAAGTTAAACAATGG 1980
CCATTGACAGAAGAAAAATAAAGCATTAGTAGAAATTTGTACAGAAATGGAAAAGGAA 2040
GGGAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACTCCAGTATTTGCCATAAAG 2100

FIG. 5

AAAAAAGACAGTACTAAATGGAGAAAATTAGTAGATTTTCAGAGAACTTAATAGGAGAACT 2160
CAAGACTTCTGGGAAGTTCAATTGGGAATACCCACATCCCGCAGGGTTAAAAAAGAAAAA 2220
TCAGTAACAGTACTGGATGTGGGTGATGCATATTTTTCAGTTCCTTAGATGAAGACTTC 2280
AGGAAGTATACTGCATTTACCATACCTAGTATAAATAATGAGACACCAGGGATTAGATAT 2340
CAGTACAATGTGCTTCCACAGGGATGGAAAGGATCACCAGCAATATTCCAAAGTAGCATG 2400
ACAAAAATCTTAGAGCCTTTTAGAAAACAAAATCCAGACATAGTTATCTATCAATACATG 2460
GATGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAAAATAGAGGAG 2520
CTGAGACAACATCTGTTGAGGTGGGGATTTACCACACCAGACAAAAAACATCAGAAAGAA 2580
CCTCCATTCCCTTTGGATGGGTTATGAACTCCATCCTGATAAATGGACAGTACAGCCTATA 2640
GTGCTGCCAGAAAAGACAGCTGGACTGTCAATGACATACAGAAGTTAGTGGGAAAATTG 2700
AATTGGGCAAGTCAGATTTATCCAGGGATTAAAGTAAGGCAATTATGTAAACTCCTTAGA 2760
GGAACCAAAGCACTAACAGAAGTAATACCACTAACAGAAGAAGCAGAGCTAGAAGTGGCA 2820
GAAAACAGAGAGATTCTAAAAGAACCAGTACATGGAGTGTATTATGACCCATCAAAGAC 2880
TTAATAGCAGAAATACAGAAGCAGGGGCAAGGCCAATGGACATATCAAATTTATCAAGAG 2940
CCATTTAAAAATCTGAAAACAGGAAAATATGCAAGAATGAGGGGTGCCCACACTAATGAT 3000
GTAAAACAATTAACAGAGGCAGTGCAAAAAATAACCACAGAAAGCATAGTAATATGGGGA 3060
AAGACTCCTAAATTTAAACTACCCATACAAAAGAAACATGGGAAACATGGTGGACAGAG 3120
TATTGGCAAGCCACCTGGATTCCCTGAGTGGGAGTTTGTTAATACCCCTCCTTTAGTGAAA 3180
TTATGGTACCAGTTAGAGAAAGAACCCATAGTAGGAGCAGAAACCTTCTATGTAGATGGG 3240
GCAGCTAGCAGGGAGACTAAATTAGGAAAAGCAGGATATGTTACTAATAGAGGAAGACAA 3300
AAAGTTGTCACCCTAACTGACACAACAAATCAGAAGACTGAATTACAAGCAATTCATCTA 3360
GCTTTGCAGGATTCGGGATTAGAAGTAAATATAGTAACAGACTCACAAATATGCATTAGGA 3420
ATCATTCAAGCACAACCAGATAAAAGTGAATCAGAGTTAGTCAATCAAATAATAGAGCAG 3480
TTAATAAAAAAGGAAAAGGTCTATCTGGCATGGGTACCAGCACACAAAGGAATTGGAGGA 3540
AATGAACAAGTAGATAAATTAGTCAGTGCTGGAATCAGGAAAATACTATTTTLAGATGGA 3600
ATAGATAAGGCCCAAGAAGACATGAGAAATATCACAAATAATTGGAGAGCAATGGCTAGT 3660
GATTTTAAACCTGCCACCTGTAGTAGCAAAGAAATAGTAGCCAGCTGTGATAAATGTCAG 3720
CTAAAAGGAGAAGCCATGCATGGACAAGTAGACTGTAGTCCAGGAATATGGCAACTAGAT 3780
TGTACACATTTAGAAGGAAAAGTTATCCTGGTAGCAGTTCATGTAGCCAGTGGATATATA 3840
GAAGCAGAAGTTATTCAGCAGAAACAGGGCAGGAAACAGCATATTTTCTTTTAAAATTA 3900
GCAGGAAGATGGCCAGTAAAAACAATACATACAGACAATGGCAGCAATTTACCAGTGCT 3960
ACGGTTAAGGCCGCTGTGGTGGGCGGGAATCAAGCAGGAATTTGGAATTCCTTACAAT 4020
CCCCAAAGTCAAGGAGTAGTAGAATCTATGAATAAAGAATTAAGAAAATTATAGGACAG 4080
GTAAGAGATCAGGCTGAACATCTTAAGACAGCAGTACAAATGGCAGTATTCATCCACAAT 4140
TTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATA 4200

FIG. 5 (continued)

GCAACAGACATACAAACTAAAGAATTACAAAAACAAATTACAAAATTCAAATTTTCGG	4260
GTTTATTACAGGGACAGCAGAAATCCACTTTGGAAAGGACCAGCAAAGCTCCTCTGGAAA	4320
GGTGAAGGGGCAGTAGTAATACAAGATAATAGTGACATAAAAGTAGTGCCAAGAAGAAAA	4380
GCAAAGATCATTAGGGATTATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAAGTAGA	4440
CAGGATGAGGATTAGAACATGGAAAAGTTTAGTAAAACACCATATGTATGTTTCAGGGAA	4500
AGCTAGGGGATGGTTTTATAGACATCACTATGAAAGCCCTCATCCAAGAATAAGTTCAGA	4560
AGTACACATCCCCTAGGGGATGCTAGATTGGTAATAACAACATATTGGGGTCTGCATAC	4620
AGGAGAAAGAGACTGGCATTGTTGGGTGAGGGAGTCTCCATAGAATGGAGGAAAAGGAGATA	4680
TAGCACACAAGTAGACCCTGAACTAGCAGACCAACTAATTCATCTGTATTACTTTGATTG	4740
TTTTTCAGACTCTGCTATAAGAAAGGCCTTATTAGGACACATAGTTAGCCCTAGGTGTGA	4800
ATATCAAGCAGGACATAACAAGGTAGGATCTCTACAATACTTGGCACTAGCAGCATTAAAT	4860
AACACCAAAAAGGGAAAGCCACCTTTGCCTAGTGTTACGAAACTGACAGAGGATAGATG	4920
GAACAAGCCCCAGAAGACCAAGGGCCACAGAGGGAGCCACACAATGAATGGACACTAGAG	4980
CTTTTAGAGGAGCTTAAGAATGAAGCTGTTAGACATTTTCCTAGGATTTGGCTCCATGGC	5040
TTAGGGCAACATATCTATGAACTTATGGGGATACTTGGGCAGGAGTGAAGCCATAATA	5100
AGAATTCTGCAACAACCTGCTGTTTATCCATTTTCAGAATTGGGTGTGACATAGCAGAAT	5160
AGGCGTTACTCAACAGAGGAGAGCAAGAAATGGAGCCAGTAGATCCTAGACTAGAGCCCT	5220
GGAAGCATCCAGGAAGTCAGCCTAAACTGCTTGTACCACTTGCTATTGTAAAAAGTGTT	5280
GCTTTCATTGCCAAGTTTGTTCATAACAAAAGCCTTAGGCATCTCCTATGGCAGGAAGA	5340
AGCGGAGACAGCGACGAAGAGCTC	

FIG. 5 (continued)

TGGAAGGGCTAATTCACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCCACCACA 60
CACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGAGTCAGATATCCAC 120
TGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGAGAAGTAAGAAGAAGCCA 180
ATAAAGGAGAGAACACCAGCTTGTTACACCTGTGAGCCTGCATGGAATTGATGACCCGG 240
AGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCACATGGCCCGAG 300
AGCTGCATCCGGAGTACTTCAAGAACTGCTGACATCGAGCTTGCTACAAGGGACTTTCCG 360
CTGGGGACTTTCCAGGGAGGCGTGGCCTGGGCGGGACTGGGGAGTGGCGAGCCCTCAGAT 420
CCTGCATATAAGCAGCTGCTTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGATCTGA 480
GCCTGGGAGCTCGAGCTCATCGAAGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAGT 540
AAGTAGTACATGTAATGCAACCTATACAAATAGCAATAGTAGCATTAGTAGTAGCAATAA 600
TAATAGCAATAGTTGTGTGGTCCATAGTAATCATAGAATATAGGAAAATATTAAGACAAA 660
GAAAATAGACAGGTTAATTGATAGACTAATAGAAAGAGCAGAAGACAGTGGCAATGAGA 720
GTGAAGGAGAAATATCAGCACTTGTGGAGATGGGGGTGGAGATGGGGCACCATGCTCCTT 780
GGGATGTTGATGATCTGTAGTGCTACAGAAAATTGTGGGTACAGTCTATTTAGGGGTA 840
CCTGTGTGGAAGGAAGCAACCACCTCTATTTTGTGCATCAGATGCTAAAGCATATGAT 900
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ACCCCACTCTGTGTTAGTTTAAAGTGCCTGATTTGAAGAATGATACTAATACCAATAGT 1140
AGTAGCGGGAGAATGATAATGGAGAAAGGAGAGATAAAAACTGCTCTTTCAATATCAGC 1200
ACAAGCAAAGAGGTAAGGTGCAGAAAGAATATGCATTTTTTTATAAACTTGATATAATA 1260
CCAATAGATAATGATACTACCAGCTATACGTTGACAAGTTGTAACACCTCAGTCATTACA 1320
CAGGCCTGTCCAAGGTATCCTTTGAGCCAATTCCCATACATTATTGTGCCCCGGCTGGT 1380
TTTGCGATTCTAAAATGTAATAATAAGACGTTCAATGGAACAGGACCATGTACAAATGTC 1440
AGCACAGTACAATGTACACATGGAATTAGGCCAGTAGTATCAACTCAACTGCTGTTAAAT 1500
GGCAGTCTAGCAGAAGAAGAGGTAGTAATTAGATCTGTCAATTTACGGACAATGCTAAA 1560
ACCATAATAGTACAGCTGAACACATCTGTAGAAATTAATTGTACAAGACCCAACAACAAT 1620
ACAAGAAAAAGTATCCAAATCCAGAGGGGACCAGGGAGAGCATTGTTACAATAGGAAAA 1680
ATAGGAAATATGAGACAAGCACATTGTAACATTAGTAGAGCAAATGGAATGCCACTTTA 1740
AAACAGATAGATAGCAAATTAAGAGAACAATTTGGAAATAATAAAACAATAATCTTTAAG 1800
CAGTCCTCAGGAGGGGACCCAGAAATTGTAACGCACAGTTTTAATTGTGGAGGGGAATTT 1860
TTCTACTGTAATTCAACACAACCTGTTAATAGTACTTGGAGTACTAAAGGGTCAAATAAC 1920
ACTGAAGGAAGTGACACAATCACCTCCCATGCAGAATAAAACAATTTATAACATGTGG 1980
CAGGAAGTAGGAAAAGCAATGTATGCCCTCCCATCAGTGGACAAATTAGATGTTTCATCA 2040
AATATTACAGGGCTGCTATTAACAAGAGATGGTGGTAATAGCAACAATGAGTCCGAGATC 2100

FIG. 6

TTCAGACCTGGAGGAGGAGATATGAGGGACAATTGGAGAAGTGAATTATATAAATATAAA 2160
GTAGTAAAAATTGAACCAFTAGGAGTAGCACCCACCAAGGCAAAGAGAAGAGTGGTGCAG 2220
AGAGAAAAAAGAGCAGTGGGAATAGGAGCTTTGTTTCCTTGGGTTCCTTGGGAGCAGCAGGA 2280
AGCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTTGTCTGGT 2340
ATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGGCCAACAGCATCTGTTGCAA 2400
CTCACAGTCTGGGGCATCAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAA 2460
AGGATCAACAGCTCCTGGGGATTTGGGGTTGCTCTGGAAAACCTATTTGCACCACTGCTG 2520
TGCCTTGAATGCTAGTTGGAGTAATAAATCTCTGGAACAGATTTGGAATAACATGACCT 2580
GGATGGAGTGGGACAGAGAAATTAACAATTACACAAGCTTAATACACTCCTTAATTGAAG 2640
AATCGCAAACCAGCAAGAAAAGAATGAACAAGAATTATTGGAATTAGATAAATGGGCAA 2700
GTTTGTGGAATTGGTTTAAACATAACAAATTGGCTGTGGTATATAAAAATTATTCATAATGA 2760
TAGTAGGAGGCTTGGTAGGTTTAAAGAATAGTTTTTGTCTGTACTTTCTATAGTGAATAGAG 2820
TTAGGCAGGGATATTCACCATTATCGTTTCAGACCCACCTCCCAAACCCGAGGGGACCCG 2880
ACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTTCGAT 2940
TAGTGAACGGATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCAGCT 3000
ACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGGAACCTCTGGGACGCA 3060
GGGGGTGGGAAGCCCTCAAATATTGGTGAATCTCCTACAGTATTGGAGTCAGGAACTAA 3120
AGAATAGTGCTGTAACTTGCTCAATGCCACAGCCATAGCAGTAGCTGAGGGGACAGATA 3180
GGGTTATAGAAGTATTACAAGCAGCTTATAGAGCCATTCGCCACATACCTAGAAGAATAA 3240
GACAGGGCTTGGAAGGATTTTGTCTATAAGATGGGTGGCAAGTGGTCAAAAAGTAGTGTG 3300
GTTGGATGGCCTGCTGTAAGGGAAAGAATGAGACGAGCTGAGCCAGCAGCAGATGGGGTG 3360
GGAGCAGTATCTCGAGACCTAGAAAAACATGGAGCAATCACAAAGTAGCAATACAGCAGCT 3420
ACCAATGCTGATTGTGCTTGGCTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTC 3480
ACACCTCAGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTT 3540
TTAAAAGAAAAGGGGGGACTGGAAGGGCTAATTCACTCCCAACGAAGACAAGATATCCTT 3600
GATCTGTGGATCCACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGG 3660
CCAGGGATCAGATATCCACTGACCTTTGGATGGTGTCTACAAGCTAGTACCAGTTGAGCCA 3720
GAGAAGATAGAAGAAGCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTG 3780
CATGGGATGGATGACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCA 3840
TTTCATCACATGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCTGACATCGAGCT 3900
TGCTACAAGGGACTTTCCGCTGGGGACTTTGCGTGGCCTGGGCGGGACTGGGGAGTGGCG 3960
AGCCCTCAGATCCTGCATATAATTTTTGCTGTACTGGGTCTCTCTGGTTAGACCAGATC 4020
TGAGCCTGGGAGCTC

FIG. 6 (continued)

TGGAAGGGCTAATTCACCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACA	60
CACAAGGCTACTTCCCTGATTAGCAGAACTACACACCAGGGCCAGGGATCAGATATCCAC	120
TGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGAGAAGTTAGAAGAAGCCA	280
ACAAAGGAGAGAACACCAGCTTGTACACCCTGTGAGCCTGCATGGAATGGATGACCCGG	240
AGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTCATCACATGGCCCGAG	300
AGCTGCATCCGGAGTACTTCAAGAACTGCTGACATCGAGCTTGCTACAAGGGACTTTCCG	360
CTGGGGACTTTCAGGGAGGCGTGGCCTGGGCGGGACTGGGGAGTGGCGAGCCCTCAGAT	420
CCTGCATATAAGCAGCTGCTTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGATCTGA	480
GCCTGGGAGCTCGAGCTCTCTCGACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGA	540
GGCGAGGGGCGGCGACTGGTGAGTACGCCAAAAATTTGACTAGCGGAGGCTAGAAGGAG	600
AGAGATGGGTGCGAGAGCGTCAGTATTAAGCGGGGAGAAATTAGATCGATGGGAAAAAAT	660
TCCGTTAAGGCCAGGGGGAAAGAAAAAATATAAATTAAAACATATAGTATGGGCAAGCAG	720
GGAGCTAGAACGATTTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACA	780
AATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAATTAGATCATTATA	840
TAATACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGATAAAAGACACCAAGGA	900
AGCTTTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAGAAAAAAGCACAGCAAGCAGC	960
AGCTGACACAGGACACAGCAGTCAGGTCAGCCAAAATTACCCTATAGTGCAGAACATCCA	1020
GGGGCAAATGGTACATCAGGCCATATCACCTAGAACTTTAAATGCATGGGTAAAAGTAGT	1080
AGAAGAGAAGGCTTTCAGCCCAGAAGTAATACCCATGTTTTTCAGCATTATCAGAAGGAGC	1140
CACCCACAAGATTTAAACACCATGCTAAACACAGTGGGGGGACATCAAGCAGCCATGCA	1200
AATGTTAAAAGAGACCATCAATGAGGAAGCTGCAGAATGGGATAGAGTACATCCAGTGCA	1260
TGCAGGGCCTATTGCACCAGGCCAGATGAGAGAACCAAGGGGAAGTGACATAGCAGGAAC	1320
TACTAGTACCCTTCAGGAACAAATAGGATGGATGACAAATAATCCACCTATCCAGTAGG	1440
AGAAATTTATAAAAGATGGATAATCCTGGGATTAATAAAAATAGTAAGAATGTATAGCCC	1500
TACCAGCATTCTGGACATAAGACAAGGACCAAAGAACCCTTTTAGAGACTATGTAGACCG	1560
GTTCTATAAAACTCTAAGAGCCGAGCAAGCTTCACAGGAGGTAAAAAATTGGATGACAGA	1620
AACCTTGTTGGTCCAAAATGCGAACCAGATTGTAAGACTATTTTAAAAGCATTGGGACC	1680
AGCGGCTACACTAGAAGAAATGATGACAGCATGTCAGGGAGTAGGAGGACCCGGCCATAA	1740
GGCAAGAGTTTTGGCTGAAGCAATGAGCCAAGTAACAAATACAGCTACCATAATGATGCA	1800
GAGAGGCAATTTTAGGAACCAAAGAAAGATGGTTAAGTGTTCATTGTGGCAAAGAAGG	1860
GCACACAGCCAGAAATTGCAGGGCCCCCTAGGAAAAAGGGCTGTTGGAAATGTGGAAAGGA	1920
AGGACACCAAATGAAAGATTGTAAGTACTGAGAGACAGGCTAATTTTTTAGGGAAGATCTGGCC	1980
TTCTTACAAGGGAAGGCCAGGGAATTTCTTCAGAGCAGACCAGAGCCAACAGCCCCACC	2040
ATTTCTTCAGAGCAGACCAGAGCCAACAGCCCCACCAGAAGAGAGCTTCAGGTCTGGGGT	2100
AGAGACAACAACCTCCCCCTCAGAAGCAGGAGCCGATAGACAAGGAACTGTATCCTTTAAC	2160

FIG. 7

TTCCCTCAGATCACTCTTTGGCAACGACCCCTCGTCACAATAAAGATAGGGGGGCAACTA	2220
AAGGAAGCTCTATTAGATACAGGAGCAGATGATACAGTATTAGAAGAAATGAGTTTGCCA	2280
GGAAGATGGAAACCAAAAATGATAGGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTAT	2340
GATCAGATACTCATAGAAATCTGTGGACATAAAGCTATAGGTACAGTATTAGTAGGACCT	2400
ACACCTGTCAACATAATTGGAAGAAATCTGTTGACTCAGATTGGTTGCACTTTAAATTTT	2460
CCCATTAGCCCTATTGAGACTGTACCAGTAAAATTAAGCCAGGAATGGATGGCCCAAAA	2520
GTTAAACAATGGCCATTGACAGAAGAAAAATAAAGCATTAGTAGAAATTTGTACAGAA	2580
ATGGAAAAGGAAGGGAAAATTTCAAAAATTGGGCCTGAGAATCCATACAATACTCCAGTA	2640
TTTGCCATAAAGAAAAAGACAGTACTAAATGGAGAAAATTAGTAGATTTTCAGAGAACTT	2700
AATAAGAGAACTCAAGACTTCTGGGAAGTTCAATTAGGAATACCACATCCCGCAGGGTTA	2760
AAAAGAAAAAATCAGTAACAGTACTGGATGTGGGTGATGCATATTTTTTCAGTTCCCTTA	2820
GATGAAGACTTCAGGAAGTATACTGCATTTACCATACCTAGTATAAACAATGAGACACCA	2880
GGGATTAGATATCAGTACAATGTGCTTCCACAGGGATGGAAAGGATCACCAGCAATATTC	2940
CAAAGTAGCATGACAAAAATCTTAGAGCCTTTTAAAAAACAAAATCCAGACATAGTTATC	3000
TATCAATACATGGATGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACA	3060
AAAATAGAGGAGCTGAGACAACATCTGTTGAGGTGGGGACTTACCACACCAGACAAAAAA	3120
CATCAGAAAGAACCCTCCATTCCTTTGGATGGGTTATGAACTCCATCCTGATAAATGGACA	3180
GTACAGCCTATAGTGCTGCCAGAAAAAGACAGCTGGACTGTCAATGACATACAGAAGTTA	3240
GTGGGGAAATTGAATTGGGCAAGTCAGATTTACCCAGGGATTAAGTAAGGCAATTATGT	3300
AAACTCCTTAGAGGAACCAAGCACTAACAGAAGTAATACCACTAACAGAAGAAGCAGAG	3360
CTAGAACTGGCAGAAAACAGAGAGATTCTAAAAGAACCAGTACATGGAGTGTATTATGAC	3420
CCATCAAAAGACTTAATAGCAGAAATACAGAAGCAGGGGCAAGGCCAATGGACATATCAA	3480
ATTTATCAAGAGCCATTTAAAAATCTGAAAACAGGAAAATATGCAAGAATGAGGGGTGCC	3540
CACACTAATGATGTAAAACAATTAACAGAGGCAGTGCAAAAAATAACCACAGAAAGCATA	3600
GTAATATGGGGAAAGACTCCTAAATTTAAACTACCCATACAAAAGGAAACATGGGAAACA	3600
TGGTGGACAGAGTATTGGCAAGCCACCTGGATTCTGAGTGGGAGTTTGTTAATACCCCT	3660
CCTTTAGTGAAATTATGGTACCAGTTAGAGAAAGAACCATAGTAGGAGCAGAAACCTTC	3720
TATGTAGATGGGGCAGCTAACAGGGAGACTAAATTAGGAAAAGCAGGATATGTTACTAAC	3780
AAAGGAAGACAAAAGGTTGTCCCCCTAACTAACACAACAAATCAGAAAATGAGTTACAA	3840
GCAATTTATCTAGCTTTGCAGGATTCAGGATTAGAAGTAAACATAGTAACAGACTCACAA	3900
TATGCATTAGGAATCATTCAAGCACACCAGATAAAAGTGAATCAGAGTTAGTCAATCAA	3960
ATAATAGAGCAGTTAATAAAAAAGGAAAAGGTCTATCTGGCATGGGTACCAGCACACAAA	4020
GGAATTGGAGGAAATGAACAAGTAGATAAATTAGTCAGTGCTGGAATCAGGAAAATACTA	4080
TTTTTAGATGGAATAGATAAGGCCCAAGATGAACATGAGAAATATCACAGTAATTGGAGA	4140
GCAATGGCTAGTGATTTTAACTGCCACCTGTAGTAGCAAAGAAATAGTAGCCAGCTGT	4200
GATAAATGTCAGCTAAAAGGAGAAGCCATGCATGGACAAGTAGACTGTAGTCCAGGAATA	4260

FIG. 7 (continued)

TGGCAACTAGATTGTACACATTTAGAAGGAAAAGTTATCCTGGTAGCAGTTCATGTAGCC 4320
AGTGGATATATAGAAGCAGAAGTTATTCAGCAGAAACAGGGCAGGAAACAGCATATTTT 4380
CTTTTAAAATTAGCAGGAAGATGGCCAGTAAAAACAATACATACAGACAATGGCAGCAAT 4440
TTCACCAGTGCTACGGTTAAGGCCCGCCTGTTGGTGGGCGGGAATCAAGCAGGAATTTGGA 4500
ATTCCCTACAATCCCCAAAGTCAAGGAGTAGTAGAATCTATGAATAAAGAATTAAAGAAA 4560
ATTATAGGACAGGTAAGAGATCAGGCTGAACATCTTAAGACAGCAGTACAAATGGCAGTA 4620
TTCATCCACAATTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGAATA 4680
GTAGACATAATAGCAACAGACATACAAATAAAGAATTACAAAAACAATTACAAAAATT 4740
CAAAATTTTCGGGTTTATTACAGGGACAGCAGAAATCCACTTTGGAAAGGACCAGCAAAG 4800
CTCCTCTGGAAAGGTGAAGGGGCAGTAGTAATACAAGATAATAGTGACATAAAAGTAGTG 4860
CCAAGAAGAAAAGCAAAGATCATTAGGGATTATGGAAAACAGATGGCAGGTGATGATTGT 4920
GTGGCAAGTAGACAGGATGAGGATTAGAACATGGAAAAGTTTAGTAAAACACCATATGTA 4980
TGTTTCAGGGAAAGCTAGGGGATGGTTTTATAGACATCACTATGAAAGCCCTCATCCAAG 5040
AATAAGTTCAGAAGTACACATCCCCTAGGGGATGCTAGATTGGTAATAACAACATATTG 5100
GGGTCTGCATACAGGAGAAAGAGACTGGCATTGGGTGAGGAGTCTCCATAGAATGGAG 5160
GAAAAGAGATATAGCACACAAGTAGACCCTGAACTAGCAGACCAACTAATTCATCTGTA 5220
TTACTTTGACTGTTTTTCAGACTCTGCTATAAGAAAGGCCTTATTAGGACACATAGTTAG 5280
CCCTAGGTGTGAATATCAAGCAGGACATAACAAGGTAGGATCTCTACAATACTTGGCACT 5340
AGCAGCATTAAATAACACCAAAAAAGATAAAGCCACCTTTGCCTAGTGTTACGAAACTGAC 5400
AGAGGATAGATGGAACAAGCCCCAGAAGACCAAGGGCCACAGAGGGAGCCACACAATGAA 5460
TGGACACTAGAGCTTTTAGAGGAGCTTAAGAATGAAGCTGTTAGACATTTTCCTAGGATT 5520
TGGCTCCATGGCTTAGGGCAACATATCTATGAACTTATGGGGATACTTGGGCAGGAGTG 5580
GAAGCCATAATAAGAATTCTGCAACAACCTGCTGTTTATCCATTTTCAGAATTGGGTGTCTG 5640
ACATAGCAGAATAGGCGTTACTCGACAGAGGAGAGCAAGAAATGGAGCCAGTAGATCCTA 5700
GACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAACCTGCTTGTACCAATTGCTATT 5760
GTAAAAGTGTTGCTTTTCATTGCCAAGTTTGTTCATAACAAAAGCCTTAGGCATCTCCT 5820
ATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAAG 5880
TTTCTCTATCAAAGCAGTAAGTAGTACATGTAATGCAACCTATACAAATAGCAATAGTAG 5940
CATTAGTAGTAGCAATAATAATAGCAATAGTTGTGTGGTCCATAGTAATCATAGAATATA 6000
GGAAAATATTAAGACAAAGAAAAATAGACAGGTTAATTGATAGACTAATAGAAAGAGCAG 6060
AAGACAGTGGCAATGAGAGTGAAGGAGAAATATCAGCACTTGTGGAGATGGGGGTGGAGA 6120
TGGGGCACCATGCTCCTTGGGATGTTGATGATCTGTAGTGCTACAGAAAAATTGTGGGTC 6180
ACAGTCTATTATGGGGTACCTGTGTGGAAGGAAGCAACCACCACTCTATTTTGTGCATCA 6240
GATGCTAAAGCATATGATACAGAGGTACATAATGTTTGGGCCACACATGCCTGTGTACCC 6300
ACAGACCCCAACCCACAAGAAGTAGTATTGGTAAATGTGACAGAAAATTTTAAACATGTGG 6360
AAAAATGACATGGTAGAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGCCTA 6420

FIG. 7 (continued)

AAGCCATGTGTAAAATTAACCCCACTCTGTGTTAGTTTAAAGTGCCTGATTTGAAGAAT 6480
GATACTAATACCAATAGTAGTAGCGGGAGAATGATAATGGAGAAAGGAGAGATAAAAAAC 6540
TGCTCTTTCAATATCAGCACAAGCATAAGAGGTAAGGTGCAGAAAGAATATGCATTTTTT 6600
TATAAACTTGATATAATACCAATAGATAATGATACTACCAGCTATACGTTGACAAGTTGT 6660
AACACCTCAGTCATTACACAGGCCTGTCCAAAGGTATCCTTTGAGCCAATTCCCATACAT 6720
TATTGTGCCCCGGCTGGTTTTGCGATTCTAAAATGTAATAATAAGACGTTCAATGGAACA 6780
GGACCATGTACAAATGTCAGCACAGTACAATGTACACATGGAATTAGGCCAGTAGTATCA 6840
ACTCAACTGCTGTTAAATGGCAGTCTAGCAGAAGAAGAGGTAGTAATTAGATCTGCCAAT 6900
TTCACAGACAATGCTAAAACCATAATAGTACAGCTGAACCAATCTGTAGAAATTAATTGT 6960
ACAAGACCCAACAACAATACAAGAAAAAGTATCCGTATCCAGAGAGGACCAGGGAGAGCA 7020
TTTGTTACAATAGGAAAAATAGGAAATATGAGACAAGCACATTGTAACATTAGTAGAGCA 7080
AAATGGAATAACACTTTAAAACAGATAGATAGCAAATTAAGAGAACAATTTGGAAATAAT 7140
AAAACAATAATCTTTAAGCAGTCCTCAGGAGGGGACCCAGAAATTGTAACGCACAGTTTT 7200
AATTGTGGAGGGGAATTTTTCTACTGTAATTCAACACAACCTGTTTAATAGTACTTGGTTT 7260
AATAGTACTTGGAGTACTAAAGGGTCAAATAACACTGAAGGAAGTGACACAATCACCTC 7320
CCATGCAGAATAAAACAAATTATAAACATGTGGCAGGAAGTAGGAAAAGCAATGTATGCC 7380
CCTCCCATCAGTGGACAAATTAGATGTTTCAATATTACAGGGCTGCTATTAACAAGA 7440
GATGGTGGTAATAGCAACAATGAGTCCGAGATCTTCAGACCTGGAGGAGGAGATATGAGG 7500
GACAATTGGAGAAGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTA 7560
GCACCCACCAAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAGAGCAGTGGGAATAGGA 7620
GCTTTGTTCCCTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACG 7680
CTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTG 7740
AGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTC 7800
CAGGCAAGAATCCTGGCTGTGAAAGATACCTAAAGGATCAACAGCTCCTGGGGATTTGG 7860
GGTTGCTCTGGAAACTCATTTCACCCTGCTGTGCCTTGGAAATGCTAGTTGGAGTAAT 7920
AAATCTCTGGAACAGATTTGGAATAACATGACCTGGATGGAGTGGGACAGAGAAATTAAC 7980
AATTACACAAGCTTAATACACTCCTTAATTGAAGAATCGCAAACCAGCAAGAAAAGAAT 8040
GAACAAGAATTATTGGAATTAGATAAATGGGCAAGTTTGTGGAATTGGTTTAACATAACA 8100
AATTGGCTGTGGTATATAAAATTATTCATAATGATAGTAGGAGGCTTGGTAGGTTAAGA 8160
ATAGTTTTTGCTGTACTTTCTGTAGTGAATAGAGTTAGGCAGGGATATTCACCATTATCG 8220
TTTCAGACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAA 8280
GGGAGAGAGAGACAGAGACAGATCCATTGATTAGTGAACGGATCCTTAGCACTTATCT 8340
GGGACGATCTGCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACTCTTGA 8400
TTGTAACGAGGATTGTGGAACCTCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGT 8460
GGAATCTCCTACAGTATTGGAGTCAGGAGCTAAAGAATAGTGCTGTTAGCTTGCTCAATG 8520
CCACAGCTATAGCAGTAGCTGAGGGGACAGATAGGGTTATAGAAGTAGTACAAGGAGCTT 8580

FIG. 7 (continued)

ATAGAGCTATTCGCCACATACCTAGAAGAATAAGACAGGGCTTGAAAGGATTTTGCTAT	8640
AAGATGGGTGGCAAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTGCTGTAAGGGAAAGA	8700
ATGAGACGAGCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTAGAAAAA	8760
CATGGAGCAATCACAAGTAGCAACACAGCAGCTAACAATGCTGATTGTGCCTGGCTAGAA	8820
GCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATG	8880
ACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAGAAAAGGGGGGACTGGAAGGG	8940
CTAATTCACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGC	9000
TACTTCCCTGATTAGCAGAACTACACACCAGGGCCAGGGATCAGATATCCACTGACCTTT	9060
GGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGAGAAGTTAGAAGAAGCCAACAAAGGA	9120
GAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGATGACCCGGAGAGAGAA	9180
GTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCACATGGCCCGAGAGCTGCAT	9240
CCGGAGTACTTCAAGAACTGCTGACATCGAGCTTGCTACAAGGGACTTTCCGCTGGGGAC	9300
TTTCCAGGAGGCGTGGCCTGGGCGGGACTGGGGAGTGGCGAGCCCTCAGATCCTGCATAT	9360
AAGGAGCTGCTTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAG	9420
CTC	

FIG. 7 (continued)

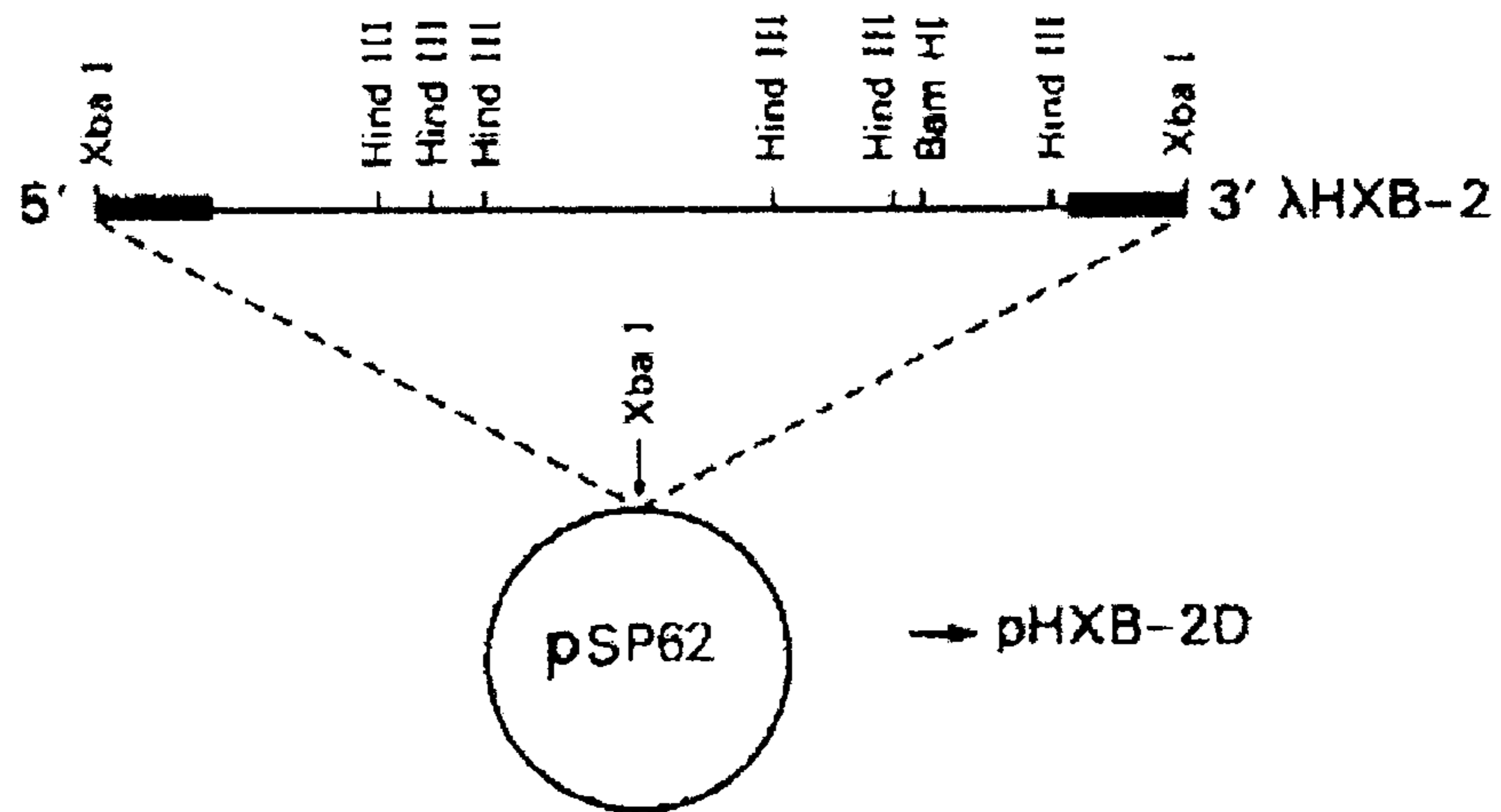


FIG. 9

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MOLECULAR CLONING OF HIV-1 FROM
IMMORTALIZED CELL LINES

REFERENCE TO RELATED APPLICATIONS

This application, Ser. No. 08/385,231, filed Feb. 8, 1995, is a file wrapper continuation of patent application Ser. No. 07/832,603, filed Feb. 12, 1992, now abandoned, which is a file wrapper continuation of patent application Ser. No. 07/160,827, filed Feb. 26, 1988, now abandoned, which is (i) a continuation-in-part of patent application Ser. No. 07/033,891, filed Apr. 3, 1987, now abandoned, which is a continuation of patent application Ser. No. 06/643,306, filed Aug. 22, 1984, now abandoned, (ii) a continuation-in-part of patent application Ser. No. 06/693,866, filed Jan. 23, 1985, pending, which is a continuation-in-part of patent application Ser. No. 06/659,339, filed Oct. 10, 1984, now abandoned, which is a continuation-in-part of patent application Ser. No. 06/643,306, filed Aug. 22, 1984, now abandoned, and (iii) a continuation-in-part of patent application Ser. No. 06/813,069, filed Dec. 24, 1985, now abandoned, the disclosures of which are incorporated herein by reference in their entirety for all purposes.

TERMINOLOGY

The causative agent of acquired immune deficiency syndrome (AIDS) has been known as human T-lymphotropic virus type III (HTLV-III) and as human immunodeficiency virus (HIV). The virus, in accord with the newer practice, will be called HIV except in some instances where a deposit relating to the organism has been made using the earlier terminology.

BRIEF DESCRIPTION OF THE INVENTION

The cultivation of viruses using molecular clones provides a dependable source of virus for study of the natural virus and for preparation of diagnostic and immunogenic products of the virus. The isolation of virus believed to be the causative agent of AIDS was reported by Barre-Sinoussi, et al. in *Science*, Vol. 220, at pages 868-870 (1983). However, no reproduction of the virus in an immortalized cell line is disclosed in that publication. HIV is highly cytopathic to the cells which it infects in nature. This is one characteristic which differentiates HIV from related retroviruses such as HTLV-I and HTLV-II. HIV is further characterized by variation of its genome in nature. Gallo, et al. discovered cell lines useful for continuous production of the virus. The use of such cell lines which are CD-4 positive cells was disclosed in U.S. patent application Ser. No. 06/652,599, which issued as U.S. Pat. No. 4,652,599. The disclosure of that patent is incorporated herein by reference. The disclosure herein provides means for producing clones of virus which are grown in the immortalized cell lines.

The infectious clones of the inventions are useful for producing specific viral proteins in both eukaryotic and prokaryotic systems for use in diagnostic evaluation and for vaccine development. The infectious clones also provide a source of homogeneous viral particles for use in evaluation of vaccines.

While HXB2 and HXB3 were shown to be non-infectious or only mildly infectious, infectious clones which have been derived therefrom are disclosed. Transfection of the derivative clones into bacteria provides a means for amplifications of the genome of these clones.

It is the object of this invention to provide a reliable source of HIV, viral particles, proteins, and antibodies by preparation

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of clones containing essentially the entire genome of the HIV. The virus or viral fragments produced in immortalized cell lines are useful as probes to detect HIV viral sequences in HIV strains isolated from patients. By use of such probes the variant strains of HIV can be studied as a means of determining source of the disease in an individual. Such determination of source is vital in evaluating means of transmission of this disease.

It is a further object of the invention to provide reliable sources of viral products for use as immunogens and diagnostic agents.

BACKGROUND OF THE INVENTION

The characterization of HIV as the causative agent of AIDS by Barre-Sinoussi, et al. [*Science*, Vol. 220 (1983)] did not provide enablement for producing the virus in vitro. However, it was discovered by workers in this laboratory that the causative agent of AIDS could be grown in immortalized CD-4 positive cell lines to provide a reliable source of the virus and viral products. The use of these products as diagnostic tools is disclosed in U.S. Pat. No. 4,520,113, which is incorporated herein by reference.

A method of cloning human T-cell leukemia-lymphoma virus (HTLV), a transforming virus which lacks both the variability and cytopathic properties of HIV, is taught in Manzari, et al., [*Proc. Natl. Acad. Sci.*, Vol. 80, pages 1574-1577 (1983)]. There is no teaching of how to clone a highly cytopathic virus of such diverse genomic structure as the HIV. To obtain a virus for cloning, it was necessary to have an infected, immortalized cell line from which to extract the virus. U.S. Pat. No. 4,652,599 to Gallo, et al. teaches such cell lines.

DESCRIPTION OF THE FIGURES

FIG. 1 is a Southern blot analysis of unintegrated DNA of HIV. No viral sequences could be detected in the undigested DNA after 4 hours. However, a major species of viral DNA of approximately 10 kb length was present in the 10, 15, 24 and 48 hour harvest representing the linear unintegrated form of the virus. A representative Southern blot of the 15 hour harvest digested with several restriction enzymes is shown in this figure. Methods: 8×10^8 fresh uninfected H9 cells were infected with concentrated supernatant from cell line H9/HTLV-III (H9/HIV) containing 4×10^{11} particles of HIV. Infected cells were divided into five Roller bottles and harvested after 4, 10, 15, 24 and 48 hours. Low molecular weight DNA was prepared using the Hirt fractionation procedure and 30 g of undigested and digested DNA were separated on a 0.8% agarose gel, transferred to nitrocellulose paper and hybridized to a HIV cDNA probe for 24 hours at 37° C. in 1×SSC, 40% formamide and 10% Dextran sulfate. cDNA was synthesized from poly(A) selected RNA prepared from doubly banded HIV virus in the presence of oligo(dT) primers. Filters were washed at 1×SSC at 65° C.

FIG. 2 is a restriction endonuclease map of two closely related HIV variants cloned from unintegrated viral DNA. Three recombinant clones (λ BH10, λ BH5 and λ BH8) were analyzed and their inserts (9 Kb, 5.5 Kb and 3.5 Kb, respectively) were mapped with the indicated enzymes. They represent two variant forms of HIV differing in three enzyme sites which are depicted in bold letters and by an asterisk. As SstI cuts the LTR of the HIV the three clones represent two full-length genomes with one LTR. A schematic map of this viral genome is shown at the bottom of the figure, although the total length of the LTR is approximate.

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FIG. 3 demonstrates HIV viral sequences in the infected cell line H9/HIV. Both variant forms of HIV were detected as integrated provirus as well as unintegrated viral DNA in the infected cell line. However, no viral sequences were found in uninfected H9 cells, uninfected HT cells nor in normal human thymus (NT).

FIG. 4 shows a sequence homology of HIV to related retroviral members of the HTLV family. A schematic restriction map of HTLV-I, HTLV-Ib and HTLV-II is drawn below indicating the length and the location of the generated fragments in respect to the corresponding genomic regions.

FIG. 5 represents the entire nucleotide sequence of the molecular clone BH5 (ATCC #40126), which is approximately one half of a cloned genomic sequence for an HIV strain on deposit with the American Type Culture Collection.

FIG. 6 represents the entire nucleotide sequence of the molecular clone BH8 (ATCC #40127), which is approximately the second half of the genomic sequence for the HIV strain noted in FIG. 5.

FIG. 7 represents the entire nucleotide sequence of the molecular clone BH10 (ATCC #40125), which is approximately the entire cloned genomic sequence of an HIV strain, different from the strain noted in FIGS. 5 and 6, on deposit with the American Type Culture Collection.

FIG. 8 shows restriction endonuclease maps of four closely related clones of HIV. λ HXB-2 and λ HXB-3 represent full-length integrated proviral forms of HIV obtained from the λ phage library of H9/HIV DNA (Example 3). These clones contain the complete provirus (thin lines) including two LTR regions plus flanking cellular sequences (heavy lines). The LTR regions are known to contain the three restriction enzyme sites Bgl II, Sst I, and Hind III, as shown, but their overall lengths are estimated. Clones λ BH-10 and λ BH-5/ λ BH-8 are shown here for comparison with λ HXB-2 and λ HXB-3 and with Southern blots of genomic DNA from other HTLV-III containing cells. It should be noted that λ BH-5/ λ BH-8 consists of two separate clones Sst I fragments (λ BH-5 and λ BH-8) which together constitute one HIV genomic equivalent but which are not necessarily derived from the same viral molecule. Also, because λ BH-10 and λ BH-5 were cloned with the restriction enzyme Sst I, they lack 5' LTR sequences as shown. Other differences in the restriction maps between these HIV clones are indicated by bold letters and asterisks, with λ BH-10 being used as a reference.

FIG. 9 shows the construction of a plasmid containing sequences of the HIV genome. A 12.7-kb XbaI fragment derived from HXB-2, a molecular clone containing about 10 kb of HIV proviral sequences, was inserted into the XbaI site in the polylinker of plasmid pSP62 to produce the plasmid clone pHXB-2D. This plasmid construct was then transfected into DH-1 bacteria and used in protoplast fusion experiments. The thin horizontal line represents HIV and the solid boxes represent flanking cellular sequences.

DETAILED DESCRIPTION OF THE INVENTION

Clones are prepared using both unintegrated DNA and integrated DNA proviral DNA. The clones of integrated DNA and unintegrated DNA are similar, but are distinguishable by differences in several restriction cleavage sites. From FIG. 8, it is shown λ BH-10 and λ BH-5/ λ BH-8 are incomplete viral clones which lack a short SstI-SstI segment of approximately 190 base pairs in the 5' LTR-leader sequence as a consequence of use of Sst I in their cloning. The λ HXB-2 and λ HXB-3 clones contain full-length integrated provirus [~10 kilobases (kb)] with cellular flanking sequences.

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Plasmids are constructed using λ HXB-2 to produce pHXB-2D A 12.7 kb XbaI fragment derived from pHXB-2D was inserted into the XbaI site in the polylinker of plasmid pSP62 to provide a plasmid suitable for transfection into the DH-1 bacteria.

Preparation of Clones λ BH10, λ BH5, and λ BH8

Example 1

Concentrated virus from the H9/HTLV-III cell line as used to infect fresh uninfected H9 cells at a multiplicity of 50 viral particles per cell and cultures were collected after 4, 10, 15, 24 and 48 hours. Extrachromosomal DNA was extracted according to the procedure of Hirt [Hirt, R., *J. Molec. Biol.* 26: 365-367 (1967)] and assayed for its content of unintegrated viral DNA using HIV cDNA as a probe. The synthesis of this cDNA was primed with oligo(dT) and reverse-transcribed from poly(A)-containing RNA of virions that had been banded twice on sucrose density gradients [Arya, et al., *Science* 225: 927-930 (1984)]. Unintegrated linear viral DNA was first detected after 10 h and was also present at the subsequent time points. (FIG. 1 shows a Southern blot of the 15-h sampling.) A band of ~10 kilobases (kb) in the undigested DNA represents the linear form of unintegrated HIV. No closed or nicked circular DNA could be detected at 10, 15 or 24 hours, but both forms were evident in small amounts at 48 hours (data not shown). The viral genome was not cleaved by XbaI, whereas SstI generated three predominant bands of 9, 5.5 and 3.5 kb (FIG. 1). These bands represent the genomes of two forms of HIV, both cut by SstI in or near the long terminal repeat (LTR), and one having an additional SstI site in the middle of its genome. The other enzymes generate a more complex pattern of restriction fragments.

FIG. 2 shows the restriction map of three clones, designated λ BH10, λ BH5 and λ BH8, which correspond in size to the three SstI fragments shown in FIG. 1. Comparison of these maps suggests that λ BH5 plus λ BH8 constitute one HIV genome, and BH10 another. The two viral forms differ in 3 of 21 mapped enzymes sites, including the internal SstI site. As expected, the phage inserts of λ BH5 and λ BH8 hybridize in high-stringency conditions (T_m -25° C.) to λ BH10 but not to each other, as analyzed by Southern blot hybridization and electron microscopic hetero-duplex analysis (data not shown). To determine the orientation of the three clones, we used as a probe a cDNA clone (C15) containing U3 and R sequences. C15 hybridized strongly to the 0.5 kb BglII fragment of λ BH10 and λ BH8, orienting this side 3'. Assuming that SstI cuts only once in the vicinity of the HIV LTR, the clones λ BH10 and λ BH5/ λ BH8 represent two complete genomic equivalents of the linear unintegrated form of HIV that vary in three restriction enzyme sites.

Methods: Low molecular weight DNA combined from the 15 and 24 hour harvest was fractionated on a 10-40% sucrose gradient. Aliquots of the fractions were electrophoresed on a 0.5% agarose gel, transferred to nitrocellulose paper and hybridized to HIV cDNA under conditions described in FIG. 1. Fractions which contained the unintegrated linear HIV genome shown by hybridization were pooled, the DNA was subsequently digested with SstI and ligated to phosphatase treated SstI arms of λ gtWes. λ B. After in vitro packaging, recombinant phages were screened for viral sequences with HIV cDNA.

Example 2

The presence of two variant forms of HIV in the original cell line was demonstrated by hybridizing the radiolabelled

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insert of λ BH10 to a Southern blot of H9/HIV genomic DNA digested with several restriction enzymes (FIG. 3); both forms were detected using SstI, which generated the expected three bands of 9, 5.5 and 3.5 kb. XbaI, which does not cut the provirus, generated a high-molecular weight smear representing polyclonal integration of the provirus, plus a band of ~10 kb. This 10-kb band was also detected in undigested H9/HIV DNA (not shown), indicating that it represents unintegrated viral DNA. The presence of unintegrated viral DNA also explains the 4- and 4.5-kb EcoRI fragments seen in both the Hirt and total cellular DNA preparations (FIGS. 1, 3). Both BglII and HindIII cut within the LTR and generate the expected internal bands. Several faint bands in addition to the expected internal bands generated by HindIII digestion, represent either defective proviruses or other variant forms of HIV present in low copy number.

Method: 10 μ g of high molecular weight DNA were digested with restriction enzymes as indicated and hybridized to nick translated phage insert from BH10 under the same conditions as described in FIG. 1.

For comparison, sub-clones of full length genomes of a prototype HTLV-I, HTLV-Ib, HTLV and GaLV (Seato strain) were digested with the following enzymes, PstI plus SstI (HTLV-I and HTLV-Ib), BamHI plus SmaI (HTLV-II) and Hind III plus SmaI plus XhoI (GaLV). Four replicate filters were prepared and hybridized for 36 hours under low stringency (8 \times SSC, 20% formamide, 10% Dextran sulfate at 37 $^{\circ}$ C.) to nick translated insert of λ BH10. Filters were then washed in 1 \times SSC at different temperatures, 22 $^{\circ}$ C. (T_m -70 $^{\circ}$ C.) filter 1, 37 $^{\circ}$ C. (T_m -56 $^{\circ}$ C.) filter 2, 50 $^{\circ}$ C. (T_m -42 $^{\circ}$ C.) filter 3 and 65 $^{\circ}$ C. (T_m -28 $^{\circ}$ C.).

FIG. 4 shows a sequence homology between HIV and other related retroviruses. Hybridization of HIV with the related HTLV family could be detected where no hybridization to GaLV was seen.

Preparation of Clones Containing Integrated Proviral DNA

Example 3

The HIV is used to infect H9 cells in accord with the method of Example 1. Preliminary analyses of Southern digests of H9/HIV DNA reveals that the virus is present in this cell line both as unintegrated DNA and as proviral DNA integrated into the cellular genome at multiple different sites. Since the HIV provirus lacks Xba I restriction sites, a genomic library was constructed by using Xba I-digested H9/HIV DNA, and this was screened with an HIV cDNA probe to obtain molecular clones of full-length integrated provirus with flanking cellular sequences. Fourteen such clones were obtained from an enriched library of 10⁶ recombinant phage, and two of these were plaque-purified and characterized. (See FIG. 8.)

To show that the restriction enzyme cleavage sites depicted in FIG. 8 for clones λ HXB-2 and λ HXB-3 are actually present in the viral DNA of HIV-infected H9 cells, DNA was digested from the H9/HIV cell line with various restriction enzymes and analyzed it by the Southern blot technique. The restriction fragments for Sst I, Eco RI, Hind III, Pst I, Bam HI, and BglII predicted from the restriction maps of λ HXB-2 and λ HXB-3 (FIG. 8) are shown to be present in the Southern blots of HIV infected cellular DNA.

Example 4

To determine whether the HIV genome contains sequences homologous to normal human DNA, the viral insert of

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λ HXB-2 (5.5 kb and 3.5 kb Sst I-Sst I fragments) was isolated, nick translated, and used to probe HIV-infected and uninfected cellular DNA. Under standard conditions of hybridization [washing conditions: 1 \times SSC (standard saline citrate), 65 $^{\circ}$ C.; annealing temperature T_m -27 C], this probe hybridizes to DNA from H9/HIV cells as well as other HIV-infected cells, but not to DNA from uninfected H9 cells, uninfected HT cells (the parent cell line from which H9 as cloned), or normal human tissues (data not shown). This finding is in agreement with previous results in which the unintegrated (replicative intermediate) form of HIV was used as probe and demonstrates that HIV, like HTLV-1 and HTLV-II, is an exogenous retrovirus lacking nucleic acid sequences derived from human DNA.

Example 5

A 12.7 kb XbaI fragment derived from λ HXB-2 is inserted into the polylinker of plasmid pSP62 to produce plasmid clone pHXB-2D (FIG. 9). The pHXB-2D is transfected into DH-1 bacteria for use in protoplast fusion experiments.

Example 6

Kinetics of cell growth and reverse transcriptase activity in cord blood mononuclear cell cultures following protoplast fusion: Mononuclear cells were prepared from cord blood samples using Ficoll Triosil and cultured for 5 days in media containing PHA. These cells were then fused with bacterial protoplasts carrying the plasmid pHXB-2D, pSV2neo or pCH-1gpt and maintained in culture at a density of 5 \times 10⁵ cells ml⁻¹ by addition of RPMI-1640 medium containing 20% fetal calf serum, 10% T-cell growth factor (inter-leukin-2) and antibiotics. Three parallel fusions using cells from different individuals were established for each plasmid. Spent medium removed from two cultures at 5, 11, 14 and 18 days after fusion was concentrated 10-fold and assayed for the presence of reverse transcriptase using standard techniques. The activity detected in each of the culture supernatants is expressed as the amount of ³H-labeled deoxyribonucleotide monophosphate (³H-dTMP) incorporated (in pmol per 0.3-ml sample) using dT₁₅·(rA)_n as the template primer.

The growth of all cultures was comparable for the first 14 days after protoplast fusion. By day 18, however, the number of viable cells in cultures transfected with pHXB-2D had fallen dramatically: there was a 10-fold and a 100-fold reduction between days 18 and 21 and 18 and 32, respectively. Cultures transfected with either pSV2neo or pCH-1gpt showed only a 4-5-fold reduction over the same time period. When supernatant from the cultures was assayed for the presence of reverse transcriptase, activity was detected exclusively in cultures transfected with pHXB-2D. These data suggest that replicating virus was present in cultures 11-18 days after fusion with pHXB-2D protoplasts.

Example 7

Expression of the HIV gag-related proteins p15 and p24 by transfected cells was demonstrated using specific monoclonal antibodies. Maximum expression was observed 18 days after transfection, when 4-11% and 5-9% of cells were reactive with antibody to p15 and p24, respectively. Virus particles were detected by electron microscopy in all cultures 14-18 days after transfection with pHXB-2D. The particles contained condensed, truncated cores, which are characteristic of HIV particles.

In time-course experiments, DNA isolated from a single culture 6, 11, 14, 18 and 31 days after transfection with pHXB-2D, was digested with BamHI and analyzed for HIV sequences. Six days after transfection, an 8.6-kb DNA fragment was detected as a faint band; 18 days after transfection it was possible to detect a 1.5-kb DNA fragment in addition to the 8.6-kb fragment. The total amount of unintegrated virus in the cultures appeared to increase, as suggested by the increase in intensity of these bands with time; this is evidence that cells originally transfected with pHXB-2D are able to produce fully infectious virus which is then transmitted within the culture.

No HIV viral sequences were detected 31 days after transfection; at this point the culture may have contained only cells which failed to be infected by HIV. This result is again consistent with the transfected DNA exerting a cytopathic effect on T cells. The finding that, at any stage, only a minor population of the transfected cells are apparently infected by the virus (<15% express viral proteins) suggests that the cytopathic effects may not result solely from direct viral infection and that secreted factors and/or other cell-to-cell interactions may play a part in the cytopathic phenomenon.

The biological materials relating to the invention have been deposited at the American Type Culture Collection, Rockville, Md., under the following accession numbers:

λBH-10	40125 (FIG. 7)
λBH-5	40126 (FIG. 5)

λBH-8	40127 (FIG. 6)
λ-HXB ₂	40231
λ-HXB ₃	40232
pHXB3	67081
pHXB-2D	67082
X10-1 (<i>E coli</i> DH-1)	67083

Upon issuance of a patent on the present invention, this deposit will continue to be viably maintained for 30 years and made available to the public without restriction, of course, consistent with the provisions of the law.

Examples of useful products are now described:

1. Viral particles and proteins may be extracted from both supernatants and whole cells.
2. Supernatant material may be purified for use in test kits for immunoblotting and immunoabsorbent tests.
3. Monoclonal antibodies may be produced which react against HIV antigens.
4. The antigens may be used as immunogens in vaccine development.

Both antibodies and antigens can be used in diagnostic kits. Both antibodies and antigens can be provided as compositions. Particularly preferred compositions of matter are solid supports having antigens of the invention adhering thereto for use in identifying antibodies to HIV proteins for use in Enzyme-linked-immunoabsorbent (ELISA) assays.

It is understood that the examples and embodiments described herein are for illustration purposes. Examples are not intended to be viewed as limitations since many obvious modifications are within the scope of one skilled in the art.

TABLE I

CLAIMS SUPPORT CHART

Claim	Support in U.S. patent application 06/643,306 filed on Aug. 22, 1984
61. (new) A method for detecting the presence of a polynucleotide comprising a human immunodeficiency virus (HIV) nucleotide sequence in a nucleic acid sample obtained from a physiological sample, which method comprises the steps of: (a) combining said nucleic acid sample with a single-stranded nucleic acid probe comprising a sequence of at least about 18 contiguous bases selected from one of the nucleotide sequences shown in FIGS. 5, 6 or 7 and complementary to said HIV genomic sequence comprised in said polynucleotide, said probe not forming a duplex with HTLV-I and -II nucleic acid sequences under conditions of stringency for hybridization under which said probe forms a duplex with said polynucleotide; and (b) determining duplex formation between said probe and nucleic acid present in said sample.	page 1, lines 28-34. discusses the use of cDNA clones of the invention to distinguish HTLV III from HTLV I and II. p. 3 line 25 to p. 4 line 8 discuss regions of homology and regions of variability between HTLV III, and HTLV I and II, that can be exploited in distinguishing between the different viruses. p. 5, lines 12-14 discusses production of a cDNA library for use in analyzing the HIV genome. p. 5 line 29 - p. 6 line 10 provides support for making probes from HIV mRNA. p. 6, lines 23-26 discusses using probes to assay viral DNA p. 7, lines 18-30 discuss using an λ phage clone in Southern analysis of restriction fragments from HIV DNA. Statement of Deposit, p. 6. p. 1 discussion relating to detection of HIV in human sera. BH10 contains an 18 base BglII-SstI restriction fragment. FIG. 4 shows that only a fraction of the HTLV-I and -II genomes hybridize to HTLV-III. Example 2, p. 8 discusses the use of stringency washes to distinguish homology between HIV, HTLV I and HTLV II. p. 3 contains a discussion relating to the presence of gag, pol and env in HTLV-III.
62. (new) The method of claim 61 wherein the probe sequence is complementary to a sequence which is part of the gag, pol or env open reading frame.	
63. (new) The method of claim 62 wherein the probe sequence is complementary to a sequence which is part of the gag open reading frame.	
64. (new) The method of claim 62 wherein the probe is complementary to a sequence which is part of the pol open reading frame.	
65. (new) The method of claim 61 wherein the probe comprises RNA.	p. 5 discussion of the use of RNA as probe/probe template.
67. (new) The method of claim 62 wherein the probe comprises RNA.	
66. (new) The method of claim 61 wherein the probe comprises DNA.	Support noted for claim 1 refers to DNA probes
68. (new) The method of claim 62 wherein the probe comprises DNA.	

TABLE I-continued

CLAIMS SUPPORT CHART

Claim	Support in U.S. patent application 06/643,306 filed on Aug. 22, 1984
<p>69. (new) A method comprising the steps of:</p> <p>(a) providing a sample suspected of containing a polynucleotide;</p> <p>(b) providing a single-stranded nucleic acid of 18-103 bases comprising a sequence of bases of at least 18 contiguous bases selected from the gag, env, or pol open reading frames of FIG. 5, 6 or 7 or the complement thereof; and</p> <p>(c) combining said sample and said single-stranded nucleic acid under hybridization conditions that (i) permit duplex formation between said single-stranded nucleic acid and either strand of viral DNA from a lambda bacteriophage selected from the group consisting of ATCC Accession no. 40143 and 40144, but (ii) do not permit duplex formation with either HTLV-I or HTLV-II genomic sequences.</p>	<p>Support noted for claim 61 also applies here.</p> <p>clone BH5 contains a HindIII-XbaI fragment that is 103 bases in length.</p> <p>p. 3 contains a discussion relating to the presence of gag, pol and env in HTLV-III.</p>
<p>70. (new) A method comprising the steps of:</p> <p>(a) providing a sample suspected of containing a polynucleotide;</p> <p>(b) providing a single-stranded nucleic acid of 32-103 bases comprising a sequence of bases of at least 32 contiguous bases selected from the gag, env, or pol open reading frames of FIG. 5, 6 or 7 or the complement thereof; and</p> <p>(c) combining said sample and said single-stranded nucleic acid under hybridization conditions that (i) permit duplex formation between said single-stranded nucleic acid and either strand of viral DNA from a lambda bacteriophage selected from the group consisting of ATCC Accession no. 40143 and 40144, but (ii) do not permit duplex formation with either HTLV-I or HTLV-II genomic sequences.</p>	<p>p. 3 contains a discussion relating to the presence of gag, pol and env in HTLV-III.</p>
<p>71. (new) The method of claim 69 or 70 wherein contiguous bases are from the gag open reading frame or the complement thereof.</p>	<p>p. 3 contains a discussion relating to the presence of gag, pol and env in HTLV-III.</p>
<p>72. (new) The method of claim 69 or 70 wherein said contiguous bases are from the env open reading frame or the complement thereof.</p>	
<p>73. (new) The method of claim 69 or 70 wherein said contiguous bases are from the pol open reading frame or the complement thereof.</p>	
<p>74. (new) The method of claim 61, 69 or 70 wherein said single-stranded nucleic acid comprises DNA and wherein said contiguous bases are within a restriction fragment produced by cleavage of the nucleic acid presented in FIG. 5, 6 or 7 using one or more restriction enzymes selected from the group consisting of SstI, HindIII, PstI, Bgl II, Kpn I, EcoRI, BamHI, HpaI, XhoI, XbaI and SmaI.</p>	<p>SstI pg. 2 and FIG. 2; HindIII pg. 4, and FIG. 2; PstI pg. 2 and FIG. 2 Bgl II pg. 7 and FIG. 2; Kpn I FIG. 2; EcoRI Page 7 and FIG. 2; BamHI Page 2 and FIG. 2; HpaI Page 2 and FIG. 2; XhoI Page 2 and FIG. 2; XbaI Page 7 and FIG. 3; and SmaI FIG. 2.</p>
<p>75. (new) The method of claim 74 wherein the single-stranded nucleic acid probe comprises one of the nucleotide sequences selected from the group consisting of: [Restriction Fragments supported in Wong-Staal specification.]</p>	<p>p. 9, lines 28-32 discusses the use of λBH10 and restriction fragments to analyze the HIV genome.</p>
<p>76. (new) The method of claim 75, wherein the single-stranded nucleic acid is 5'-CTTAAAGACCAATGACTTACAAGGCAGCTGTA -3'.</p>	<p>p. 6, lines 23-26 discusses using cDNA to detect viral DNA</p> <p>p. 7, lines 18-30 and p. 9 lines 15-26 discuss using an λ phage clone in Southern analysis of restriction fragments from HIV DNA.</p> <p>32 nucleotide restriction fragment present in BH8.</p> <p>Nucleotide sequence of claim 76 nucleic acid is a 32 nucleotide KpnI-BglII restriction fragment from clone BH8</p> <p>Support cited for claim 1 is applicable here.</p> <p>See also support for claim 76.</p>
<p>77. (new) A method for detecting the presence of a polynucleotide comprising a human immunodeficiency virus (HIV) nucleotide sequence in a nucleic acid sample obtained from a physiological sample, which method comprises the steps of:</p>	
<p>(a) combining said nucleic acid sample with a single-stranded nucleic acid probe comprising a sequence of at least about 32 contiguous bases selected from one of the nucleotide sequences shown in FIGS. 5, 6 or 7 and complementary to said HIV genomic sequence comprised in said polynucleotide, said probe not forming a duplex with HTLV-I and -II nucleic acid sequences under conditions of stringency for hybridization under which said probe forms a duplex with said polynucleotide; and</p>	
<p>(b) determining duplex formation between said probe and nucleic acid present in said sample.</p>	
<p>78. (new) The method of claim 61, wherein the single-stranded nucleic acid probe is 5'- GATCTGAGCCTGGGAGCT-3'.</p>	<p>18 base BglII-SstI restriction fragment of BH10.</p>
<p>79. (new) The method of any of claims 69-73 wherein said single-stranded nucleic acid comprises RNA.</p>	<p>Discussion on p. 5 relating to the use of RNA as probe/probe template.</p>
<p>80. (new) The method of any of claims 69-73 wherein said single-stranded nucleic acid comprises DNA.</p>	
<p>81. (new) The method of claim 79 wherein said single-stranded nucleic acid further comprises a label.</p>	
<p>82. (new) The method of claim 80 wherein said single-stranded nucleic acid further comprises a label.</p>	
<p>83. (new) The method of claims 69-73, wherein said sample is a human sample.</p>	<p>p. 1, lines 28-34. discusses the use of cDNA clones of the invention to distinguish HTLV III from HTLV I and II.</p> <p>Original claim 5 discusses radiolabels.</p> <p>FIGS. 1, 3 and 4 show assays using labeled DNA.</p> <p>p5, lines 24-26 discuss labeled probes, and p. 7 lines 18-22 discuss nick translation.</p> <p>p. 1 contains a discussion of the use of probes to detect HTLV-III in human sera.</p>

TABLE I-continued

CLAIMS SUPPORT CHART

Claim	Support in U.S. patent application 06/643,306 filed on Aug. 22, 1984
84. (new) The method of claim 83, wherein said human sample is blood, lymph or saliva.	
85. (new) The method of claims 84, wherein said sample is blood.	
86. (new) A method for detecting the presence of a polynucleotide comprising a human immunodeficiency virus (HIV) nucleotide sequence in a nucleic acid sample, the method comprising the steps of: (a) combining said nucleic acid sample with a single-stranded nucleic acid probe hybridizing under stringent conditions to an HIV nucleotide sequence present in a nucleic acid deposit selected from the group consisting of H9/HTLV-III cell line, CRL 8543; BH10, ATCC #40125; BH8, ATCC #40127; and BH5, ATCC #40126, said probe not forming a duplex with HTLV-I and -II nucleic acid sequences under conditions of stringency for hybridization that allow said probe to form a duplex with said polynucleotide; and (b) determining duplex formation between said probe and said nucleic acid present in said sample.	page 1, lines 28-34. discusses the use of cDNA clones of the invention to distinguish HTLV III from HTLV I and II. p. 3 line 25 to p. 4 line 8 discuss regions of homology and regions of variability between HTLV III, and HTLV I and II, that can be exploited in distinguishing between the different viruses. p. 5, lines 12-14 discusses production of a cDNA library for hybridization analysis of the HIV genome. p. 5 line 29 - p. 6 line 10 provides support for making cDNA probes from HIV mRNA. p. 6, lines 23-26 discusses using cDNA probes to assay viral DNA p. 7, lines 18-30 discuss using an λ phage clone in southern analysis of restriction fragments from HIV DNA by Southern blot. Statement of Deposit, p. 6. Example 2, p. 8 discusses the use of stringency washes to distinguish homology between HIV, HTLV I and HTLV II. p. 5 lines 23-28 discuss hybridizing cDNA sequences to genomic restriction fragments of HIV. p. 5, lines 12-14 discusses production of a cDNA library for hybridization analysis of the HIV genome. p. 5 lines 23-28 discuss hybridizing cDNA sequences to genomic restriction fragments of HIV. See also, FIG. 4 (restriction map) and p. 3, line 30 to p. 4 line 3. Support noted for claim 1 refers to DNA probes
87. (new) The method of claim 86, wherein the nucleic acid probe is a restriction fragment.	
88. (new) The method of claim 86, wherein the probe sequence is complementary to a sequence that is part of the gag, pol or env coding regions.	
89. (new) The method of claim 86, wherein the probe comprises DNA.	
90. (new) The method of claim 87, wherein the probe comprises DNA.	
91. (new) A method comprising the steps of: (a) providing a sample suspected of containing a polynucleotide; (b) providing a single-stranded HIV cDNA; and, (c) combining said sample and said single-stranded HIV cDNA under hybridization conditions that (i) permit duplex formation between said HIV cDNA and either nucleotide strand from a lambda bacteriophage selected from the group consisting of λ BH10, λ BH5 and λ BH8, but (ii) do not permit duplex formation with either HTLV-I or HTLV-II genomic sequences.	p. 1, lines 28-34. discusses the use of cDNA clones of the invention to distinguish HTLV III from HTLV I and II. p. 3 line 25 to p. 4 line 8 discuss regions of homology and regions of variability between HTLV III, and HTLV I and II, that can be exploited in distinguishing between the different viruses. p. 5, lines 12-14 discusses production of a cDNA library for hybridization analysis of the HIV genome. p. 5 line 29 - p. 6 line 10 provides support for making cDNA probes from HIV mRNA. p. 9, lines 28-32 discusses the use of the λ BH10 clone and HIV genomic restriction fragments in hybridization studies. p. 6, lines 23-26 discusses using cDNA probes to assay viral DNA p. 7, lines 18-30 and p. 9 lines 15-26 discuss using an λ phage clone in Southern analysis of restriction fragments from HIV DNA. Statement of Deposit, p. 6. Example 2, p. 8 discusses the use of stringency washes to distinguish homology between HIV, HTLV I and HTLV II. FIGS. 2 and 3 provide restriction maps that would allow one of skill to identify probes to particular genomic regions. p. 9, lines 28-32 specifically discusses use probes to analyze restriction fragments. Also note discussion from p. 5, line 29 to p. 6, line 26 regarding the use of cDNA probes See also, FIG. 4 and p. 3, line 30 to p. 4 line 3.
92. (new) The method of claim 91, wherein said single-stranded nucleic acid is an SstI fragment or complement thereof.	
93. (new) The method of claim 91 wherein said single-stranded nucleic acid is a HindIII fragment or complement thereof.	
94. (new) The method of claim 91 wherein said single-stranded nucleic acid comprises DNA and hybridizes to a restriction fragment generated by treating an HIV genomic nucleic acid with HindIII and BamHI.	FIGS. 2 and 3 provide restriction maps that would allow one of skill to identify probes to particular genomic regions. p. 9, lines 28-32 specifically discusses hybridization analysis using restriction fragments. Also note discussion from p. 5, line 29 to p. 6, line 26 regarding the use of cDNA sequences as probes. See also, FIG. 4 and p. 3, line 30 to p. 4 line 3. FIGS. 2 and 3 provide restriction maps that would allow one of skill to identify probes to particular genomic regions. p. 9, lines 28-32 specifically discusses use probes to analyze restriction fragments. Also note discussion from p. 5, line 29 to p. 6, line 26 regarding the use of cDNA probes See also, FIG. 4 and p. 3, line 30 to p. 4 line 3.
95. (new) The method of any of claims 91-94 wherein said single-stranded nucleic acid comprises DNA.	
96. (new) The method of claim 95 wherein said single-stranded nucleic acid further comprises a label.	p. 1, lines 28-34. discusses the use of cDNA clones of the invention to distinguish HTLV III from HTLV I and II. Original claim 5 discusses radiolabels. FIGS. 1, 3 and 4 show assays using labeled DNA. p5, lines 24-26 discuss labeled probes, and p. 7 lines 18-22 discuss nick translation.
97. (new) The method of claim 92 wherein said single-stranded nucleic acid comprises DNA and wherein said contiguous bases are within the gag open reading frame.	FIGS. 2 and 3 provide restriction maps that would allow one of skill to identify probes to particular genomic regions. p. 9, lines 28-32 specifically discusses hybridization analysis using restriction fragments. Also note discussion from p. 5, line 29 to p. 6, line 26 regarding the use of cDNA sequences as probes. See also, FIG. 4 and p. 3, line 30 to p. 4 line 3.

TABLE I-continued

CLAIMS SUPPORT CHART

Claim	Support in U.S. patent application 06/643,306 filed on Aug. 22, 1984
98. (new) The method of claim 97 wherein said single-stranded nucleic acid further comprises a label.	Original claim 5 discusses radiolabels. FIGS. 1, 3 and 4 show assays using labeled DNA. p5, lines 24-26 discuss labeled probes, and p. 7 lines 18-22 discuss nick translation.
99. (new) A method for detecting the presence of a polynucleotide comprising a human immunodeficiency virus (HIV) genomic sequence in a nucleic acid sample obtained from a physiological sample, which method comprises the steps of: (a) combining said nucleic acid sample with a single-stranded nucleic acid probe comprising a sequence of at least about 20 contiguous bases selected from the nucleotide sequences shown in FIGS. 5-7 and complementary to said HIV genomic sequence comprised in said polynucleotide, said probe not forming a duplex with HTLV-I and -II nucleic acid sequences under conditions of stringency for hybridization under which said probe forms a duplex with said polynucleotide; and (b) determining duplex formation between said probe and nucleic acid present in said sample.	page 1, lines 28-34. discusses the use of cDNA clones of the invention to distinguish HTLV III from HTLV I and II. p. 3 line 25 to p. 4 line 8 discuss regions of homology and regions of variability between HTLV III, and HTLV I and II, that can be exploited in distinguishing between the different viruses. p. 5, lines 12-14 discusses production of a cDNA library for use in hybridization studies of the HIV genome. p. 5 line 29 - p. 6 line 10 provides support for making cDNA probes from HIV mRNA. p. 6, lines 23-26 discusses using cDNA sequences for use in hybridization studies of the HIV genome. p. 7, lines 18-30 discuss using an λ phage clone in Southern analysis of restriction fragments from HIV DNA. Statement of Deposit, p. 6. p. 1 discussion relating to detection of HIV in human sera. BH10 contains an 18 base BglII-SstI restriction fragment. p. 3 contains a discussion relating to the presence of gag, pol and env in HTLV-III.
100. (new) The method of claim 99 wherein the probe sequence is complementary to a sequence which is part of the gag, pol or env open reading frame.	
101. (new) The method of claim 100 wherein the probe sequence is complementary to a sequence which is part of the gag open reading frame.	
102. (new) The method of claim 100 wherein the probe is complementary to a sequence which is part of the pol open reading frame.	
103. (new) The method of claim 99 wherein the probe comprises RNA.	p. 5 discussion of the use of RNA as probe/probe template.
105. (new) The method of claim 100 wherein the probe comprises RNA.	
104. (new) The method of claim 99 wherein the probe comprises DNA.	Support noted for claim 1 refers to DNA probes
106. (new) The method of claim 100 wherein the probe comprises DNA.	
107. (new) A method comprising the steps of: (a) providing a sample suspected of containing a polynucleotide; (b) providing a single-stranded nucleic acid of 20-100 bases comprising a sequence of bases of at least 20 contiguous bases selected from the gag, env, or pol open reading frames; and (c) combining said sample and said single-stranded nucleic acid under hybridization conditions that (i) permit duplex formation between said single-stranded nucleic acid and either strand of viral DNA from a lambda bacteriophage selected from the group consisting of ATCC Accession no. 40143 and 40144, but (ii) do not permit duplex formation with either HTLV-I or HTLV-II genomic sequences.	Support noted for claim 61 also applies here. p. 3 contains a discussion relating to the presence of gag, pol and env in HTLV-III.
108. (new) The method of any of claims 107 wherein said single-stranded nucleic acid comprises RNA.	p. 5 discussion of the use of RNA as probe/probe template. Support noted for claim 1 refers to DNA probes
109. (new) The method of any of claims 107 wherein said single-stranded nucleic acid comprises DNA.	
110. (new) The method of claim 108 wherein said single-stranded nucleic acid further comprises a label.	Original claim 5 discusses radiolabels. FIGS. 1, 3 and 4 show assays using labeled DNA. p5, lines 24-26 discuss labeled probes, and p. 7 lines 18-22 discuss nick translation. The paragraph spanning pages 5 and 6 discusses chemically synthesizing DNA using NaOH, an RNA template, and restriction enzymes.
111. (new) The method of claim 109 wherein said single-stranded nucleic acid further comprises a label.	
112. (new) The method of claim 108 wherein said single-stranded nucleic acid is chemically synthesized at least in part.	
113. (new) The method of claim 109 wherein said single-stranded nucleic acid is chemically synthesized at least in part.	
114. (new) The method of claim 110 wherein said single-stranded nucleic acid is chemically synthesized at least in part.	
115. (new) The method of claim 111 wherein said single-stranded nucleic acid is chemically synthesized at least in part.	
116. (new) The method of claims 99 or 101 wherein said sample is a human sample.	p. 1 contains a discussion of the use of probes to detect HTLV-III in human sera.
117. (new) The method of claim 116 wherein said human sample is blood, lymph or saliva.	
118. (new) The method of claims 99 or 101 wherein said sample is blood, lymph or saliva.	

TABLE II-continued

(BH 5 and 8 v. LUCIW)
89.8% identity

BH 5	AGAAGGAGAGAGA--TGGGTGCGAGAGCGTCAGTATTAAGCGGGGAGAATTAGATCGAT					

Licuw,	AGAAGGAGAGAGAGATGGGTGCGAGAGCGTCGGTATTAAGCGGGGAGAATTAGATAAAT					
	780	790	800	810	820	830
		660	670	680	690	700
BH 5	GGGAAAAAATTCGGTTAAGGCCAGGGGAAAGAAAAATATAAATTTAAAACATATAGTAT					

Licuw,	GGGAAAAAATTCGGTTAAGGCCAGGGGAAAGAAAAATATAAGTTAAAACATATAGTAT					
	840	850	860	870	880	890
		720	730	740	750	760
BH 5	GGGCAAGCAGGGAGCTAGAACGATTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAG					

Licuw,	GGGCAAGCAGGGAGCTAGAACGATTCGCAGTCAATCCTGGCCTGTTAGAAACATCAGAAG					
	900	910	920	930	940	950
		780	790	800	810	820
BH 5	GCTGTAGACAAATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTA					

Licuw,	GCTGCAGACAAATATTGGGACAGCTACAGCCATCCCTTCAGACAGGATCAGAAGAACTTA					
	960	970	980	990	1000	1010
		840	850	860	870	880
BH 5	GATCATTATATAATACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGATAAAAG					

Licuw,	GATCATTATATAATACAGTAGCAACCCTCTATTGTGTACATCAAAGGATAGATGATAAAAG					
	1020	1030	1040	1050	1060	1070
		900	910	920	930	940
BH 5	ACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAGAAAAAGCAC					

Licuw,	ACACCAAGGAAGCTTTAGAGAAGATAGAGGAAGAGCAAAACAAAAGTAAGAAAAAGGCAC					
	1080	1090	1100	1110	1120	1130
		960	970	980	990	1000
BH 5	AGCAAGCAGCAGCTGA-----CACAGGACACAGCAGTCAGGTCAGCCAAAATTACCCTA					

Licuw,	AGCAAGCAGCAGCTGCAGCTGGCACAGGAAACAGCAGCCAGGTCAGCCAAAATTACCCTA					
	1140	1150	1160	1170	1180	1190
		1010	1020	1030	1040	1050
BH 5	TAGTGCAGAACATCCAGGGCAAATGGTACATCAGGCCATATCACCTAGAACTTTAAATG					

Licuw,	TAGTGCAGAACCTACAGGGCAAATGGTACATCAGGCCATATCACCTAGAACTTTAAATG					
	1200	1210	1220	1230	1240	1250
		1070	1080	1090	1100	1110
BH 5	CATGGGTAAAAGTAGTAGAAGAGAAGGCTTTCAGCCAGAAGTAATACCCATGTTTTTCAG					

Licuw,	CATGGGTAAAAGTAGTAGAAGAAAAGGCTTTCAGCCAGAAGTAATACCCATGTTTTTCAG					
	1260	1270	1280	1290	1300	1310
		1130	1140	1150	1160	1170
BH 5	CATTATCAGAAGGAGCCACCCACAAGATTTAAACACCATGCTAAACACAGTGGGGGGAC					

Licuw,	CATTATCAGAAGGAGCCACCCACAAGATTTAAACACCATGCTAAACACAGTGGGGGGAC					
	1320	1330	1340	1350	1360	1370
		1190	1200	1210	1220	1230
BH 5	ATCAAGCAGCCATGCAAATGTTAAAAGAGACCATCAATGAGGAAGCTGCAGAATGGGATA					

Licuw,	ATCAAGCAGCCATGCAAATGTTAAAAGAGACTATCAATGAGGAAGCTGCAGAATGGGATA					
	1380	1390	1400	1410	1420	1430
		1250	1260	1270	1280	1290
BH 5	GAGTGCATCCAGTGCATGCAGGGCCATCGCACCAGGCCAGATGAGAGAACCAAGGGGAA					

Licuw,	GAGTGCATCCAGTGCATGCAGGGCCATTCGCACCAGGCCAAATGAGAGAACCAAGGGGAA					
	1440	1450	1460	1470	1480	1490
		1310	1320	1330	1340	1350
BH 5	GTGACATAGCAGGAACACTACTAGTACCCTTCAGGAACAAATAGGATGGATGACAAATAATC					

Licuw,	GTGACATAGCAGGAACACTACTAGTACCCTTCAGGAACAAATAGGATGGATGACAAATAATC					
	1500	1510	1520	1530	1540	1550

TABLE II-continued

(BH 5 and 8 v. LUCIW)
89.8% identity

	3650	3660	3670	3680	3690	3700
BH 5	GGAGTTTGT	TAATACCCCT	CTTTAGT	GAAATTAT	GGTACCAGT	TAGAGAAAGA
	GGAGTTTGT	CAATACCCCT	CCCTTAGT	GAAATTAT	GGTACCAGT	TAGAGAAAGA
Licuw,	3800	3810	3820	3830	3840	3850
	3710	3720	3730	3740	3750	3760
BH 5	AGTAGGAGC	AGAAACCTT	CTATGTAG	ATGGGGCAG	CTAGCAGGG	AGACTAAAT
	AGTAGGAGC	AGAAACTTT	CTATGTAG	ATGGGGCAG	CTAATAGGG	AGACTAAAT
Licuw,	3860	3870	3880	3890	3900	3910
	3770	3780	3790	3800	3810	3820
BH 5	AGCAGGATAT	GTTACTAAT	AGAGGAAG	CAAAAAAGT	TGTCACCCT	AACTGACACA
	AGCAGGATAT	GTTACTGAC	AGAGGAAG	CAAAAAAGT	TGTCTCCAT	AGCTGACACA
Licuw,	3920	3930	3940	3950	3960	3970
	3830	3840	3850	3860	3870	3880
BH 5	TCAGAAGACT	GAAATACA	AGCAATTC	CATCTAGCT	TTGCAGGAT	TCGGGATTAG
	TCAGAAGACT	GAAATACA	AGCAATTC	CATCTAGCT	TTGCAGGAT	TCGGGATTAG
Licuw,	3980	3990	4000	4010	4020	4030
	3890	3900	3910	3920	3930	3940
BH 5	TATAGTAAC	AGACTCACA	ATATGCAT	TAGGAATC	ATTCAAGCA	CAACCAGATA
	CATAGTAAC	AGACTCACA	ATATGCAT	TAGGAATC	ATTCAAGCA	CAACCAGATA
Licuw,	4040	4050	4060	4070	4080	4090
	3950	3960	3970	3980	3990	4000
BH 5	ATCAGAGTT	AGTCAATA	AATAGAGC	AGTTAATA	AAAAAAGG	AAAAGGTCT
	ATCAGAGTT	AGTCAATA	AATAGAGC	AGTTAATA	AAAAAAGG	AAAAGGTCT
Licuw,	4100	4110	4120	4130	4140	4150
	4010	4020	4030	4040	4050	4060
BH 5	ATGGGTACC	AGCACACA	AAAGGAAT	TGGAGGAA	TGAACAAG	TAGATAAAT
	ATGGGTACC	AGCACACA	AAAGGAAT	TGGAGGAA	TGAACAAG	TAGATAAAT
Licuw,	4160	4170	4180	4190	4200	4210
	4070	4080	4090	4100	4110	4120
BH 5	TGGAATCAG	GAAAATACT	ATTTTTAG	ATGGAATAG	ATAAGGCC	CAAGAAGAC
	TGGAATCAG	GAAAAGTACT	ATTTTTGA	ATGGAATAG	ATAAGGCC	CAAGAAGAC
Licuw,	4220	4230	4240	4250	4260	4270
	4130	4140	4150	4160	4170	4180
BH 5	ATATCACA	AATTTGGAG	AGCAATGG	CTAGTGAT	TTAACCTG	CCACCTGTAG
	ATATCACA	GTAAATTTGG	AGAGCAAT	GGCTAGTG	ATTTAACCT	GCCACCTGTAG
Licuw,	4280	4290	4300	4310	4320	4330
	4190	4200	4210	4220	4230	4240
BH 5	AGAAATAGT	AGCCAGCT	GTGATAA	TGTCAGCT	AAAAGGAG	AAGCCATGC
	AGAAATAGT	AGCCAGCT	GTGATAA	TGTCAGCT	AAAAGGAG	AAGCCATGC
Licuw,	4340	4350	4360	4370	4380	4390
	4250	4260	4270	4280	4290	4300
BH 5/8	AGACTGTAG	TCCAGGAAT	ATGGCAACT	AGATTGTAC	ACATTTAG	AAGAAAAGT
	AGACTGTAG	TCCAGGAAT	ATGGCAACT	AGATTGTAC	ACATCTAGA	AAGAAAATT
Licuw,	4400	4410	4420	4430	4440	4450
	4310	4320	4330	4340	4350	4360
BH 5	GGTAGCAGT	TCATGTAG	CCAGTGG	ATATATAG	AAGCAGAAG	TATTCAGC
	GGTAGCAGT	TCATGTAG	CCAGTGG	ATATATAG	AAGCAGAAG	TATTCAGC
Licuw,	4460	4470	4480	4490	4500	4510

TABLE II-continued

(BH 5 and 8 v. LUCIW)
89.8% identity

	5860	5870	5880	5890	5900	5910
BH 8	GAGCTCATCGAAGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAGTAAGTAGTACATGT					
	::::	::	::::	::::	::::	::::
Licuw, ---	CTCAG-GA--CAGTCAGACTCATCAAGTTTCTCTATCAAAGCAGTAAGTAGTAAATGT					
	6010	6020	6030	6040	6050	
	5920	5930	5940	5950	5960	5970
BH 8	AATGCAACCTATACAAATA--GCAATAGTAGCATTAGTAGTAGCAATAATAATAGCAAT					
	::::	::	::::	::::	::::	::::
Licuw,	AATGCAATCTTTACAAATATTAGCAATAGTATCATTAGTAGTAGTAGCAATAATAATAGCAAT					
	6060	6070	6080	6090	6100	6110
	5980	5990	6000	6010	6020	6030
BH 8	AGTTGTGTGGTCCATAGTAATCATAGAATATAGGAAAATATTAAGACAAAGAAAAATAGA					
	::::	::::	::::	::::	::::	::::
Licuw,	AGTTGTGTGGACCATAGTACTCATAGAATATAGGAAAATATTAAGACAAAGAAAA-TAGA					
	6120	6130	6140	6150	6160	6170
	6040	6050	6060	6070	6080	6090
BH 8	CAGGTTAATTGATAGACTAATAGAAAGAGCAGAAGACAGTGGCAATGAGAGTGAAGGAGA					
	::::	::::	::::	::::	::::	::::
Licuw,	CAGATTAATTGATAGAATAAGAGAAAAAGCAGAAGACAGTGGCAATGAAAGTGAAGGGGA					
	6180	6190	6200	6210	6220	6230
	6100	6110	6120	6130	6140	
BH 8	-----AATATCAGCACTTGTGGAGATGGGGTGGAGATGGGGCACCATGCTCCTTG					
	:	::::	::::	::::	::::	::::
Licuw,	CCAGGAGGAATTATCAGCACTTGTGGAGATGGG-----GCACCTTGCTCCTTG					
	6240	6250	6260	6270	6280	
	6150	6160	6170	6180	6190	6200
BH 8	GGATGTTGATGATCTGTAGTGCTACAGAAAATTGTGGGTACAGTCTATTTAGGGGTAC					
	::::	::::	::::	::::	::::	::::
Licuw,	GGATGTTGATGATCTGTAGTGCTACAGAAAATTGTGGGTACAGTCTATTTAGGGGTAC					
	6290	6300	6310	6320	6330	6340
	6210	6220	6230	6240	6250	6260
BH 8	CTGTGTGGAAGGAAGCAACCACCCTCTATTTTGTGCATCAGATGCTAAAGCATATGATA					
	::::	::::	::::	::::	::::	::::
Licuw,	CTGTGTGGAAGAAGCAACTACCCTCTATTTTGTGCATCAGATGCTAGAGCATATGATA					
	6350	6360	6370	6380	6390	6400
	6270	6280	6290	6300	6310	6320
BH 8	CAGAGGTACATAATGTTTGGGCCACACATGCTGTGTACCCACAGACCCCAACCCACAAG					
	::::	::::	::::	::::	::::	::::
Licuw,	CAGAGGTACATAATGTTTGGGCCACACATGCTGTGTACCCACAGACCCCAACCCACAAG					
	6410	6420	6430	6440	6450	6460
	6330	6340	6350	6360	6370	6380
BH 8	AAGTAGTATTGGTAAATGTGACAGAAAATTTAACATGTGGAAAAATGACATGGTAGAAC					
	::::	::::	::::	::::	::::	::::
Licuw,	AAGTAGTATTGGGAAATGTGACAGAAAATTTAACATGTGGAAAAATAACATGGTAGAAC					
	6470	6480	6490	6500	6510	6520
	6390	6400	6410	6420	6430	6440
BH 8	AGATGCATGAGGATATAATCAGTTTATGGGATCAAAGCCTAAAGCCATGTGTAATAATTA					
	::::	::::	::::	::::	::::	::::
Licuw,	AGATGCAGGAGGATATAATCAGTTTATGGGATCAAAGCCTAAAGCCATGTGTAATAATTA					
	6530	6540	6550	6560	6570	6580
	6450	6460	6470	6480	6490	6500
BH 8	CCCCACTCTGTGTTAGTTTAAAGTGCACTGATTTGAAGAATGATACTAATAACCAATAGTA					
	::::	::::	::::	::::	::::	::::
Licuw,	CCCCACTCTGTGTTACTTTAAATTGCACTGATTTGGGGAAGGCTACTAATAACCAATAGTA					
	6590	6600	6610	6620	6630	6640
	6510	6520	6530	6540	6550	6560
BH 8	GTAGCGGGAGAATGATAATGGAGAAAGGAGAGATAAAAACTGCTCTTCAATATCAGCA					
	:::	:::	:::	::::	::::	::::
Licuw,	GTAATTGGAAGAAGAAATA--AAAGGAGAAATAAAAACTGCTCTTCAATATCACCA					
	6650	6660	6670	6680	6690	6700
	6570	6580	6590	6600	6610	6620
BH 8	CAAGCAAAGAGGTAAGGTGCAGAAAGAATATGCATTTTTTATAAACTTGATATAATAC					
	::::	::::	::::	::::	::::	::::
Licuw,	CAAGCATAAGAGATAAGATTGAGAAAGAAAATGCATTTTTTCGTAACTTGATGTAGTAC					
	6710	6720	6730	6740	6750	6760

TABLE II-continued

		(BH 5 and 8 v. LUCIW) 89.8% identity					
BH 8	6630	6640	6650	6660	6670		
	CAATAGATAATGATA-----CTACCAGCTATAC-----GTTGACAAGTTGTAACA						
Licuw,	CAATAGATAATGCTAGTACTACTACCAACTATACCAACTATAGGTTGATACATTGTAACA						
	6770	6780	6790	6800	6810	6820	
BH 8	6680	6690	6700	6710	6720	6730	
	CCTCAGTCATTACACAGGCCTGTCCAAAGGTATCCTTTGAGCCAATTCCCATACATTATT						
Licuw,	GATCAGTCATTACACAGGCCTGTCCAAAGGTATCCTTTGAGCCAATTCCCATACATTATT						
	6830	6840	6850	6860	6870	6880	
BH 8	6740	6750	6760	6770	6780	6790	
	GTGCCCCGGCTGGTTTTGCGATTCTAAAATGTAATAATAAGACGTTCAATGGAACAGGAC						
Licuw,	GTACCCCGCTGGTTTTGCGATTCTAAAGTGAATAATAAAACGTTCAATGGAAAAGGAC						
	6890	6900	6910	6920	6930	6940	
BH 8	6800	6810	6820	6830	6840	6850	
	CATGTACAAATGTCAGCACAGTACAATGTACACATGGAATTAGGCCAGTAGTATCAACTC						
Licuw,	CATGTACAAATGTCAGCACAGTACAATGTACACATGGAATTAGGCCAATAGTGTCAACTC						
	6950	6960	6970	6980	6990	7000	
BH 8	6860	6870	6880	6890	6900		
	AACTG-CTGTTAAATGGCAGTCTAGCAGAAGAAGAGGTAGTAATTAGATCTGTCAATTTTC						
Licuw,	AACTGTCTGTTAAATGGCAGTCTAGCAGAAGAAGAGGTAGTAATTAGATCTGACAATTTTC						
	7010	7020	7030	7040	7050	7060	
BH 8	6910	6920	6930	6940	6950	6960	
	ACGGACAATGCTAAAACCATAATAGTACAGCTGAAACACATCTGTAGAAATTAATTGTACA						
Licuw,	ACGAACAATGCTAAAACCATAATAGTACAGCTGAATGAATCTGTAGCAATTAAGTGTACA						
	7070	7080	7090	7100	7110	7120	
BH 8	6970	6980	6990	7000	7010	7020	
	AGACCCAACAACAATAACAAGAAAAAGTATCCAATCCAGAGGGGACCAGGGAGAGCATTTC						
Licuw,	AGACCCAACAACAATAACAAGAAAAAGTATCTATATA-----GGACCAGGGAGAGCATTTC						
	7130	7140	7150	7160	7170		
BH 8	7030	7040	7050	7060	7070	7080	
	GTTACAATAGGAAAAATA--GGAAATATGAGACAAGCACATTGTAACATTAGTAGAGCA						
Licuw,	CATACAACAGGAAGAATAATAGGAGATATAAGAAAAGCACATTGTAACATTAGTAGAGCA						
	7180	7190	7200	7210	7220	7230	
BH 8	7090	7100	7110	7120	7130	7140	
	AAATGGAATGCCACTTTAAAACAGATAGATAGCAAATTAAGAGAACAATTTGGAAATAAT						
Licuw,	CAATGGAATAACACTTTAGAACAGATAGTTAAAAAATTAAGAGAACAATTTGGGAATAAT						
	7240	7250	7260	7270	7280	7290	
BH 8	7150	7160	7170	7180	7190	7200	
	AAAACAATAATCTTTAAGCAGTCCTCAGGAGGGGACCCAGAAATTGTAACGCACAGTTTT						
Licuw,	AAAACAATAGTCTTTAATCAATCCTCAGGAGGGGACCCAGAAATTGTAATGCACAGTTTT						
	7300	7310	7320	7330	7340	7350	
BH 8	7210	7220	7230	7240	7250	7260	
	AATTGTGGAGGGGAATTTTCTACTGTAATCAACACAACACTGTTTAATAGTACTTGGAGT						
Licuw,	AATTGTAGAGGGGAATTTTCTACTGTAATCAACACAACACTGTTTAATAATACATGGAG-						
	7360	7370	7380	7390	7400	7410	
BH 8	7270	7280	7290	7300	7310		
	ACTAAAGGGTCAAATAACACTGAAGGAAGT-----GACACAATCACCCCTCCCATGC						
Licuw,	-----GTTAAATCACACTGAAGGAAGTAAAGGAAATGACACAATCATACTCCCATGT						
	7420	7430	7440	7450	7460		

TABLE II-continued

(BH 5 and 8 v. LUCIW)
89.8% identity

	8800	8810	8820	8830	8840	8850
BH 8	TGCTTGGCTAGAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACC					
					
Licuw,	TGCCTGGCTAGAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCAGACCTCAGGTACC					
	8950	8960	8970	8980	8990	9000

	8860	8870	8880	8890	8900	8910
BH 8	TTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGG					
					
Licuw,	TTTAAGACCAATGACTTACAAGGCAGCTTTAGATATTAGCCACTTTTTAAAAGAAAAGGG					
	9010	9020	9030	9040	9050	9060

	8920	8930	8940	8950	8960	8970
BH 8	GGGACTGGAAGGGCTAATTCACCTCCAACGAAGACAAGATATCCTTGATCTGTGGATCCA					
					
Licuw,	GGGACTGGAAGGGCTAATTTGGTCCCAAAGACAAGAGATCCTTGATCTGTGGATCTA					
	9070	9080	9090	9100	9110	9120

	8980	8990	9000	9010	9020	9030
BH 8	CCACACACAAGGCTACTTCCCTGATTGGCAGAATTACACACCAGGGCCAGGGATCAGATA					
					
Licuw,	CCACACACAAGGCTACTTCCCTGATTGGCAGAATTACACACCAGGGCCAGGGATCAGATA					
	9130	9140	9150	9160	9170	9180

	9040	9050	9060	9070	9080	9090
BH 8	TCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGAGAAGATAGAAGA					
					
Licuw,	TCCACTGACCTTTGGATGGTGCTTCAAGCTAGTACCAGTTGAGCCAGAGAAGGTAGAAGA					
	9190	9200	9210	9220	9230	9240

	9100	9110	9120	9130	9140	9150
BH 8	AGCCAATAAAGGAGAGAACACCAGCTTGTACACCTGTGAGCCTGCATGGGATGGATGA					
					
Licuw,	GGCCAATGAAGGAGAGAACA-AGCTTGTACACCTATGAGCCTGCATGGGATGGAGGA					
	9250	9260	9270	9280	9290	9300

	9160	9170	9180	9190	9200	9210
BH 8	CCCTGAGAGAGAAGTGTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTCATCACATGGC					
					
Licuw,	CGCGGAGAAAGAAGTGTAGTGTGGAGGTTTGACAGCAAACCTAGCATTTCATCACATGGC					
	9310	9320	9330	9340	9350	9360

	9220	9230	9240	9250	9260	9270
BH 8	CCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCTGACATCGAGCTTGTACAAGGGACT					
					
Licuw,	CCGAGAGCTGCATCCGGAGTACTACAAAGACTGCTGACATCGAGCTTCTACAAGGGACT					
	9370	9380	9390	9400	9410	9420

	9280	9290	9300	9310	9320	9330
BH 8	TTCCGCTGGGACTTT-----GCGTGGCCTGGGCGGGACTGGGGAGTGGCGAGCCCT					
					
Licuw,	TTCCGCTGGGACTTTCCAGGGAGGCGTGGCCTGGGCGGGACTGGGGAGTGGCGT-CCCT					
	9430	9440	9450	9460	9470	

	9340	9350	9360	9370	9380	
BH 8	CAGATCCTGCATATAA-----TTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGA					
					
Licuw,	CAGATGCTGCATATAAGCAGCTGTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGA					
	9480	9490	9500	9510	9520	9530

	9390					
BH 8	TCTGAGCCTGGGAGCTC-----					
					
Licuw,	TCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCT					
	9540	9550	9560	9570	9580	9590

Licuw,	TGCCTTGAGTGCTTCAAGTAGTGTGTGCCGCTCTGTTGTGTGACTCTGGTAACTAGAGAT					
	9600	9610	9620	9630	9640	9650

Licuw,	CCCTCAGACCCTTTTAGTCAGTGTGGAAAAATCTCTAGCAG				
	9660	9670	9680	9690	9700

TABLE III

(BH 10 v. LUCIW)
90.0% identity

	10	20	30	40	50	
BH 10	-TGGAAGGGCTAATTC	ACTCCAACGAAGCA	AGATATCCTTGATCT	GTGGATCTACCAC		
	
Licuw,	CTGGAAGGGCTAATTT	GGTCCCAAAGAAGCA	AGAGATCCTTGATCT	GTGGATCTACCAC		
	10	20	30	40	50	60
60	70	80	90	100	110	
BH 10	ACACAAGGCTACTTCC	TGATTAGCAGAACTAC	ACACCAGGGCCAGGGAT	CAGATATCCA		
	
Licuw,	ACACAAGGCTACTTCC	TGATTGGCAGAACTAC	ACACCAGGGCCAGGGAT	CAGATATCCA		
	70	80	90	100	110	120
120	130	140	150	160	170	
BH 10	CTGACCTTTGGATGGT	GCTACAAGCTAGTACC	AGTTGAGCCAGAGAAGT	TAGAAGAAGCC		
	
Licuw,	CTGACCTTTGGATGGT	GCTTCAAGCTAGTACC	AGTTGAGCCAGAGAAGT	TAGAAGAAGCC		
	130	140	150	160	170	180
180	190	200	210	220	230	
BH 10	AACAAAGGAGAGAAC	ACCAGCTTGTTACACC	CTGTGAGCCTGCATGG	AATGGATGACCCG		
	
Licuw,	AATGAAGGAGAGAAC	ACAGCTTGTTACACC	CTATGAGCCTGCATGG	GATGGAGGACGCG		
	190	200	210	220	230	240
240	250	260	270	280	290	
BH 10	GAGAGAGAAGTGT	TAGAGTGGAGTTT	GACAGCCGCCTAGCAT	TTTCATCACATGG	CCCGA	
	
Licuw,	GAGAAAGAAGTGT	TAGTGTGGAGTTT	GACAGCAAAGT	AGCATTTTCATCAC	ATGGCCCGA	
	250	260	270	280	290	300
300	310	320	330	340	350	
BH 10	GAGCTGCATCCGAGT	ACTTCAAGAAGT	CTGACATCGAGCTT	GCTACAAGGGACT	TTTCC	
	
Licuw,	GAGCTGCATCCGAGT	ACTTCAAGAAGT	CTGACATCGAGCTT	TCTACAAGGGACT	TTTCC	
	310	320	330	340	350	360
360	370	380	390	400	410	
BH 10	GCTGGGGACTTTCC	AGGGAGGCGTGGCCT	GGGCGGGACTGGGG	AGTGGCGAGCCCT	CAGA	
	
Licuw,	GCTGGGGACTTTCC	AGGGAGGCGTGGCCT	GGGCGGGACTGGGG	AGTGGCGT-CCCT	CAGA	
	370	380	390	400	410	
420	430	440	450	460	470	
BH 10	TCCTGCATATAAGCAG-	CTGCTTTTTGCCTGT	ACTGGGTCTCTCTGGT	TAGACCAGATCT		
	:	
Licuw,	TGCTGCATATAAGCAG	ACTGCTTTTTGCCTGT	ACTGGGTCTCTCTGGT	TAGACCAGATCT		
	420	430	440	450	460	470
480	490					
BH 10	GAGCCTGGGAGCTC-	-----				
					
Licuw,	GAGCCTGGGAGCTC	TCTGGCTAACTAGGGA	ACCCTGCTTAAGCCT	CAATAAAGCTTGC		
	480	490	500	510	520	530

Licuw,	CTTGAGTGCTTCAAGT	AGTGTGCCCCGTCT	GTTGTGTGACTCTGG	TAACTAGAGATCCC		
	540	550	560	570	580	590

Licuw,	TCAGACCCTTTTAGT	CAGTGTGGAAAAAT	CTCTAGCAGTGGCG	CCCGAACAGGGAC	GCGA	
	600	610	620	630	640	650
500	510	520	530			
BH 10	-----GAGCTCTCT	CGACGCAGGACTCGG	CTTGTGAAGCGCGCA			
	
Licuw,	AAGCGAAAGTAGAAC	CAGAGGAGCTCTCT	CGACGCAGGACTCGG	CTTGTGAAGCGCGCA		
	660	670	680	690	700	710
540	550	560	570	580	590	
BH 10	CGGCAAGAGGCGAGG	GGCGGCGACTGGT	GAGTACGCCAAAAAT	TTTGACTAGCGGAGG	CT	
	:	
Licuw,	CAGCAAGAGGCGAGG	GGCGGCGACTGGT	GAGTACGCCAA--	TTTTGACTAGCGGAGG	CT	
	720	730	740	750	760	770

TABLE III-continued

		(BH 10 v. LUCIW) 90.0% identity					
BH 10		2870	2880	2890	2900	2910	2920
		AGGATCACCAGCAATATTCCAAAGTAGCATGACAAAAATCTTAGAGCCTTTTAAAAACA					
Licuw,		AGGATCACCAGCAATATTCCAAAGTAGCATGACAAAAATCTTAGAGCCTTTTAAAAACA					
		3020	3030	3040	3050	3060	3070
BH 10		2930	2940	2950	2960	2970	2980
		AAATCCAGACATAGTTATCTATCAATACATGGATGATTTGTATGTAGGATCTGACTTAGA					
Licuw,		GAATCCAGACATAGTTATCTATCAATACATGGATGATTTGTATGTAGGATCTGACTTAGA					
		3080	3090	3100	3110	3120	3130
BH 10		2990	3000	3010	3020	3030	3040
		AATAGGGCAGCATAGAACAAAAATAGAGGAGCTGAGACAACATCTGTTGAGGTGGGGACT					
Licuw,		AATAGGGCAGCATAGAACAAAAATAGAGGAACTGAGACAGCATCTGTTGAGGTGGGGATT					
		3140	3150	3160	3170	3180	3190
BH 10		3050	3060	3070	3080	3090	3100
		TACCACACCAGACAAAAACATCAGAAAGAACCTCCATTCTTTGGATGGGTATGAAC					
Licuw,		TACCACACCAGACAAAAACATCAGAAAGAACCTCCATTCTTTGGATGGGTATGAAC					
		3200	3210	3220	3230	3240	3250
BH 10		3110	3120	3130	3140	3150	3160
		CCATCCTGATAAATGGACAGTACAGCCTATAGTGCTGCCAGAAAAAGACAGCTGGACTGT					
Licuw,		CCATCCTGATAAATGGACAGTACAGCCTATAATGCTGCCAGAAAAAGACAGCTGGACTGT					
		3260	3270	3280	3290	3300	3310
BH 10		3170	3180	3190	3200	3210	3220
		CAATGACATACAGAAGTTAGTGGGAAATGAATTGGGCAAGTCAGATTTACCCAGGGAT					
Licuw,		CAATGACATACAGAAGTTAGTGGGAAATGAATTGGGCAAGTCAGATTTATGCAGGGAT					
		3320	3330	3340	3350	3360	3370
BH 10		3230	3240	3250	3260	3270	3280
		TAAAGTAAAGCAATTATGTAAACTCCTTAGAGGAACCAAAGCACTAACAGAAGTAATACC					
Licuw,		TAAAGTAAAGCAATTATGTAAACTCCTTAGAGGAACCAAAGCACTAACAGAAGTAATACC					
		3380	3390	3400	3410	3420	3430
BH 10		3290	3300	3310	3320	3330	3340
		ACTAACAGAAGAAGCAGAGCTAGAACTGGCAGAAAACAGAGAGATTCTAAAAGAACCAGT					
Licuw,		ACTAACAGAAGAAGCAGAGCTAGAACTGGCAGAAAACAGGGAGATTCTAAAAGAACCAGT					
		3440	3450	3460	3470	3480	3490
BH 10		3350	3360	3370	3380	3390	3400
		ACATGGAGTGTATTATGACCCATCAAAGACTTAATAGCAGAAATACAGAAGCAGGGGCA					
Licuw,		ACATGAAGTATATTATGACCCATCAAAGACTTAGTAGCAGAAATACAGAAGCAGGGGCA					
		3500	3510	3520	3530	3540	3550
BH 10		3410	3420	3430	3440	3450	3460
		AGGCCAATGGACATATCAAATTTATCAAGAGCCATTTAAAAATCTGAAAACAGGAAAATA					
Licuw,		AGGCCAATGGACATATCAAATTTATCAAGAGCCATTTAAAAATCTGAAAACAGGAAAGTA					
		3560	3570	3580	3590	3600	3610
BH 10		3470	3480	3490	3500	3510	3520
		TGCAAGAATGAGGGGTGCCACACTAATGATGTAAAACAATTAACAGAGGCAGTGCAAAA					
Licuw,		TGCAAGGATGAGGGGTGCCACACTAATGATGTAAAACAGTTAACAGAGGCAGTGCAAAA					
		3620	3630	3640	3650	3660	3670
BH 10		3530	3540	3550	3560	3570	3580
		AATAACCACAGAAAGCATAGTAATATGGGGAAAGACTCCTAAATTTAAACTACCCATACA					
Licuw,		AGTATCCACAGAAAGCATAGTAATATGGGGAAAGATTCTAAATTTAAACTACCCATACA					
		3680	3690	3700	3710	3720	3730

TABLE III-continued

		(BH 10 v. LUCIW) 90.0% identity					
BH 10		3590	3600	3610	3620	3630	3640
		AAAGGAAACATGGGAAACATGGTGGACAGAGTATTGGCAAGCCACCTGGATTCTGAGTG					
Licuw,		AAAGGAAACATGGGAAAGCATGGTGGATGGAGTATTGGCAAGCTACCTGGATTCTGAGTG					
		3740	3750	3760	3770	3780	3790
BH 10		3650	3660	3670	3680	3690	3700
		GGAGTTTGTTAATACCCCTCCTTTAGTGAAATTTATGGTACCAGTTAGAGAAAGAACCCAT					
Licuw,		GGAGTTTGTCAATACCCCTCCCTTAGTGAAATTTATGGTACCAGTTAGAGAAAGAACCCAT					
		3800	3810	3820	3830	3840	3850
BH 10		3710	3720	3730	3740	3750	3760
		AGTAGGAGCAGAAACCTTCTATGTAGATGGGGCAGCTAACAGGGAGACTAAATTAGGAAA					
Licuw,		AGTAGGAGCAGAAACTTTCTATGTAGATGGGGCAGCTAATAGGGAGACTAAATTAGGAAA					
		3860	3870	3880	3890	3900	3910
BH 10		3770	3780	3790	3800	3810	3820
		AGCAGGATATGTTACTAACAAAGGAAGACAAAAGTTGTCCCCCTAACTAACACAACAAA					
Licuw,		AGCAGGATATGTTACTGACAGAGGAAGACAAAAGTTGTCTCCATAGCTGACACAACAAA					
		3920	3930	3940	3950	3960	3970
BH 10		3830	3840	3850	3860	3870	3880
		TCAGAAAACAGTGTACAAGCAATTTATCTAGCTTTGCAGGATTCAGGATTAGAAGTAAA					
Licuw,		TCAGAAGACTGAATTACAAGCAATTCATCTAGCTTTGCAGGATTCGGGATTAGAAGTAAA					
		3980	3990	4000	4010	4020	4030
BH 10		3890	3900	3910	3920	3930	3940
		CATAGTAACAGACTCACAATATGCATTAGGAATCATTCAAGCACAACCAGATAAAAGTGA					
Licuw,		CATAGTAACAGACTCACAATATGCATTAGGAATCATTCAAGCACAACCAGATAAGAGTGA					
		4040	4050	4060	4070	4080	4090
BH 10		3950	3960	3970	3980	3990	4000
		ATCAGAGTTAGTCAATCAAATAATAGAGCAGTTAATAAAAAAGGAAAAGGTCTATCTGGC					
Licuw,		ATCAGAGTTAGTCAATCAAATAATAGAGCAGTTAATAAAAAAGGAAAAGGTCTACCTGGC					
		4100	4110	4120	4130	4140	4150
BH 10		4010	4020	4030	4040	4050	4060
		ATGGGTACCAGCACACAAAGGAATTGGAGGAAATGAACAAGTAGATAAATTAGTCAGTGC					
Licuw,		ATGGGTACCAGCACACAAAGGAATTGGAGGAAATGAACAAGTAGATAAATTAGTCAGTGC					
		4160	4170	4180	4190	4200	4210
BH 10		4070	4080	4090	4100	4110	4120
		TGGAATCAGGAAAATACTATTTTTAGATGGAATAGATAAGGCCCAAGATGAACATGAGAA					
Licuw,		TGGAATCAGGAAAGTACTATTTTTGAATGGAATAGATAAGGCCCAAGAAAGCATGAGAA					
		4220	4230	4240	4250	4260	4270
BH 10		4130	4140	4150	4160	4170	4180
		ATATCACAGTAATTGGAGAGCAATGGCTAGTGATTTAACCTGCCACCTGTAGTAGCAA					
Licuw,		ATATCACAGTAATTGGAGAGCAATGGCTAGTGATTTAACCTGCCACCTGTAGTAGCAA					
		4280	4290	4300	4310	4320	4330
BH 10		4190	4200	4210	4220	4230	4240
		AGAAATAGTAGCCAGCTGTGATAAATGTCAGCTAAAAGGAGAAGCCATGCATGGACAAGT					
Licuw,		AGAAATAGTAGCCAGCTGTGATAAATGTCAGCTAAAAGGAGAAGCCATGCATGGACAAGT					
		4340	4350	4360	4370	4380	4390
BH 10		4250	4260	4270	4280	4290	4300
		AGACTGTAGTCCAGGAATATGGCAACTAGATTGTACACATTTAGAAGGAAAAGTTATCCT					
Licuw,		AGACTGTAGTCCAGGAATATGGCAACTAGATTGTACACATCTAGAAGGAAAATTTATCCT					
		4400	4410	4420	4430	4440	4450
BH 10		4310	4320	4330	4340	4350	4360
		GGTAGCAGTTCATGTAGCCAGTGGATATATAGAAGCAGAAGTTATCCAGCAGAAACAGG					
Licuw,		GGTAGCAGTTCATGTAGCCAGTGGATATATAGAAGCAGAAGTTATCCAGCAGAGACAGG					
		4460	4470	4480	4490	4500	4510

TABLE III-continued

(BH 10 v. LUCIW)
90.0% identity

	5860	5870	5880	5890	5900	5910
BH 10	TCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAGTAAGTAGTACATGTAATGCA					
	::: : ::					
Licuw,	TCAGGACAGTCAGACTCATCAAGCTTCTCTATCAAAGCAGTAAGTAGTAAATGTAATGCA					
	6010	6020	6030	6040	6050	6060
	5920	5930	5940	5950	5960	5970
BH 10	ACCTATACAAATA--GCAATAGTAGCATTAGTAGTAGCAATAATAATAGCAATAGTTGT					
	: : : :::::::::: : :::::::::: : :::::::::: : :::::::::: : ::::::::::					
Licuw,	ATCTTTACAAATATTAGCAATAGTATCATTAGTAGTAGTAGCAATAATAGCAATAGTTGT					
	6070	6080	6090	6100	6110	6120
	5980	5990	6000	6010	6020	6030
BH 10	GTGGTCCATAGTAATCATAGAATATAGGAAAATATTAAGACAAAGAAAAATAGACAGGTT					
	::: : :::::::::: : :::::::::: : :::::::::: : :::::::::: : ::::::::::					
Licuw,	GTGGACCATAGTACTCATAGAATATAGGAAAATATTAAGACAAAGAAA-TAGACAGATT					
	6130	6140	6150	6160	6170	6180
	6040	6050	6060	6070	6080	
BH 10	AATTGATAGACTAATAGAAAGAGCAGAAGACAGTGGCAATGAGAGTGAAGGAGA-----					
	::: : :::::::::: : :::::::::: : :::::::::: : :::::::::: : ::::::::::					
Licuw,	AATTGATAGAATAAGAGAAAAGCAGAAGACAGTGGCAATGAAAGTGAAGGGGACCAGGA					
	6190	6200	6210	6220	6230	6240
	6090	6100	6110	6120	6130	6140
BH 10	---AATATCAGCACTTGTGGAGATGGGGTGGAGATGGGGCACCATGCTCCTTGGGATGT					
	: : : :::::::::: : :::::::::: : :::::::::: : :::::::::: : ::::::::::					
Licuw,	GGAATTATCAGCACTTGTGGAGATGGGG-----CACCTTGCTCCTTGGGATGT					
	6250	6260	6270		6280	6290
	6150	6160	6170	6180	6190	6200
BH 10	TGATGATCTGTAGTGCTACAGAAAATTGTGGGTCACAGTCTATTATGGGGTACCTGTGT					
	::: : :::::::::: : :::::::::: : :::::::::: : :::::::::: : ::::::::::					
Licuw,	TGATGATCTGTAGTGCTACAGAAAATTGTGGGTCACAGTTTATTATGGAGTACCTGTGT					
	6300	6310	6320	6330	6340	6350
	6210	6220	6230	6240	6250	6260
BH 10	GGAAGGAAGCAACCACCTCTATTTTGTGCATCAGATGCTAAAGCATATGATACAGAGG					
	::: : :::::::::: : :::::::::: : :::::::::: : :::::::::: : ::::::::::					
Licuw,	GGAAAGAAGCAACTACCCTCTATTTTGTGCATCAGATGCTAGAGCATATGATACAGAGG					
	6360	6370	6380	6390	6400	6410
	6270	6280	6290	6300	6310	6320
BH 10	TACATAATGTTTGGGCCACACATGCCTGTGTACCCACAGACCCCAACCCACAAGAAGTAG					
	::: : :::::::::: : :::::::::: : :::::::::: : :::::::::: : ::::::::::					
Licuw,	TACATAATGTTTGGGCCACACATGCCTGTGTACCCACAGACCCCAACCCACAAGAAGTAG					
	6420	6430	6440	6450	6460	6470
	6330	6340	6350	6360	6370	6380
BH 10	TATTGGTAAATGTGACAGAAAATTTAACATGTGGAAAATGACATGGTAGAACAGATGC					
	::: : :::::::::: : :::::::::: : :::::::::: : :::::::::: : ::::::::::					
Licuw,	TATTGGGAAATGTGACAGAAAATTTAACATGTGGAAAATAACATGGTAGAACAGATGC					
	6480	6490	6500	6510	6520	6530
	6390	6400	6410	6420	6430	6440
BH 10	ATGAGGATATAATCAGTTTATGGGATCAAAGCCTAAAGCCATGTGTAATAAATTAACCCAC					
	: : : :::::::::: : :::::::::: : :::::::::: : :::::::::: : ::::::::::					
Licuw,	AGGAGGATATAATCAGTTTATGGGATCAAAGCCTAAAGCCATGTGTAATAAATTAACCCAC					
	6540	6550	6560	6570	6580	6590
	6450	6460	6470	6480	6490	6500
BH 10	TCTGTGTTAGTTTAAAGTGCCTGATTTGAAGAATGATACTAATACCAATAGTAGTAGCG					
	::: : :::::::::: : :::::::::: : :::::::::: : :::::::::: : ::::::::::					
Licuw,	TCTGTGTTACTTTAAATTGCCTGATTTGGGAAGGCTACTAATACCAATAGTAGTAATT					
	6600	6610	6620	6630	6640	6650
	6510	6520	6530	6540	6550	6560
BH 10	GGAGA-ATGATAATGGAGAAAGGAGAGATAAAAACTGCTCTTTCAATATCAGCACAAGC					
	::: : : : : : : :::::::::: : :::::::::: : :::::::::: : ::::::::::					
Licuw,	GGAAAGAAGA-AATA--AAAGGAGAAATAAAAACTGCTCTTTCAATATCACCACAAGC					
	6660	6670	6680	6690	6700	

TABLE III-continued

(BH 10 v. LUCIW)
90.0% identity

	8810	8820	8830	8840	8850	8860
BH 10	TGTGCCTGGCTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTA					

Licuw,	TGTGCCTGGCTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCAGACCTCAGGTA					
	8940	8950	8960	8970	8980	8990
	8870	8880	8890	8900	8910	8920
BH 10	CCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTAAAAGAAAAG					

Licuw,	CCTTTAAGACCAATGACTTACAAGGCAGCTTTAGATATTAGCCACTTTTAAAAGAAAAG					
	9000	9010	9020	9030	9040	9050
	8930	8940	8950	8960	8970	8980
BH 10	GGGGGACTGGAAGGGCTAATTCCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATC					

Licuw,	GGGGGACTGGAAGGGCTAATTTGGTCCCAAAGAAGACAAGAGATCCTTGATCTGTGGATC					
	9060	9070	9080	9090	9100	9110
	8990	9000	9010	9020	9030	9040
BH 10	TACCACACACAAGGCTACTTCCCTGATTAGCAGAATAACACACCAGGGCCAGGGATCAGA					

Licuw,	TACCACACACAAGGCTACTTCCCTGATTGGCAGAATTACACACCAGGGCCAGGGATCAGA					
	9120	9130	9140	9150	9160	9170
	9050	9060	9070	9080	9090	9100
BH 10	TATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGAGAAGTTAGAA					

Licuw,	TATCCACTGACCTTTGGATGGTGCTTCAAGCTAGTACCAGTTGAGCCAGAGAAGTTAGAA					
	9180	9190	9200	9210	9220	9230
	9110	9120	9130	9140	9150	9160
BH 10	GAAGCCAACAAAGGAGAGAACACCAGCTTGTACACCCTGTGAGCCTGCATGGAATGGAT					
	::	::	::	::	::	::
Licuw,	GAGGCCAATGAAGGAGAGAACAA-AGCTTGTACACCCTATGAGCCTGCATGGGATGGAG					
	9240	9250	9260	9270	9280	9290
	9170	9180	9190	9200	9210	9220
BH 10	GACCCGGAGAGAGAAGTGTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTATCACATG					
	::	::	::	::	::	::
Licuw,	GACCCGGAGAGAAGTGTAGTGTGGAGGTTTGACAGCAAACCTAGCATTTATCACATG					
	9300	9310	9320	9330	9340	9350
	9230	9240	9250	9260	9270	9280
BH 10	GCCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCTGACATCGAGCTTCTACAAGGGA					

Licuw,	GCCCCGAGAGCTGCATCCGGAGTACTACAAGAACTGCTGACATCGAGCTTCTACAAGGGA					
	9360	9370	9380	9390	9400	9410
	9290	9300	9310	9320	9330	9340
BH 10	CTTTCCGCTGGGGACTTTCCAGG-AGGCGTGGCCTGGGCGGGACTGGGGAGTGGCGAGCC					

Licuw,	CTTTCCGCTGGGGACTTTCCAGGGAGGCGTGGCCTGGGCGGGACTGGGGAGTGGCGT-CC					
	9420	9430	9440	9450	9460	9470
	9350	9360	9370	9380	9390	9400
BH 10	CTCAGATCCTGCATATAAGGAGCTGCTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCA					

Licuw,	CTCAGATGCTGCATATAAGCAGCTGCTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCA					
	9480	9490	9500	9510	9520	9530
	9410	9420				
BH 10	GATCTGAGCCTGGGAGCTC-----					
					
Licuw,	GATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAG					
	9540	9550	9560	9570	9580	9590
BH 10	-----					
Licuw,	CTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAG					
	9600	9610	9620	9630	9640	9650
BH 10	-----					
Licuw,	ATCCCTCAGACCCTTTTAGTCAGTGTGGAAAAATCTCTAGCAG					
	9660	9670	9680	9690	9700	

What we claim is:

1. A composition comprising a duplex formed between a Human Immunodeficiency Virus Type-1 (HIV-1) nucleic acid and a single-stranded nucleic acid of at least 18 contiguous nucleotides comprising a nucleotide sequence selected from the group consisting of:

- (i) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₂ having ATCC Accession No. 40231;
- (ii) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₃ having ATCC Accession No. 40232;
- (iii) a nucleotide sequence of either strand of viral DNA from clone BH10 having ATCC Accession No. 40125;
- (iv) a nucleotide sequence of either strand of viral DNA from clone BH5 having ATCC Accession No. 40126;
- (v) a nucleotide sequence of either strand of viral DNA from clone BH8 having ATCC Accession No. 40127;
- (vi) a nucleotide sequence of either strand of viral DNA from clone pHXB3 having ATCC Accession No. 67081;
- (vii) a nucleotide sequence of either strand of viral DNA from clone pHXB-2D having ATCC Accession No. 67082;
- (viii) a nucleotide sequence of either strand of viral DNA from *E. coli* clone X10-1 having ATCC Accession No. 67083; and

(ix) an HIV-1 nucleotide sequence from the H9/HTLV-III cell line having ATCC Accession No. CRL 8543;

wherein the single-stranded nucleic acid does not form a duplex with HTLV-I and HTLV-II nucleic acids under conditions of stringency for hybridization under which the single-stranded nucleic acid forms a duplex with the HIV-1 nucleic acid;

wherein the duplex is outside of a mammalian cell and outside of an HIV-1 particle;

wherein the duplex comprises a double-stranded region of at least 18 contiguous nucleotides and a single-stranded region on either side of the double-stranded region that is longer than the double-stranded region; and

wherein the single-stranded nucleic acid comprises a label.

2. The composition of claim 1, wherein the single-stranded nucleic acid comprises a nucleotide sequence which is part of an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or part of an HIV-1 long terminal repeat region.

3. The composition of claim 1, wherein the single-stranded nucleic acid is selected from the group consisting of:

- (i) a single-stranded nucleic acid consisting of from 18 to 103 nucleotides comprising a nucleotide sequence of at least 18 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 env open reading frame, an HIV-1 pol open reading frame or from an HIV-1 long terminal repeat region;
- (ii) a single-stranded nucleic acid consisting of from 32 to 103 nucleotides comprising a nucleotide sequence of at least 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 env open reading frame, an HIV-1 pol open reading frame or from an HIV-1 long terminal repeat region;
- (iii) a single-stranded nucleic acid consisting of from 20 to 100 nucleotides comprising a nucleotide sequence of at least 20 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 env open reading frame, an HIV-1 pol open reading frame or from an HIV-1 long terminal repeat region;

(iv) a single-stranded nucleic acid comprising a nucleotide sequence of at least about 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 env open reading frame, an HIV-1 pol open reading frame or from an HIV-1 long terminal repeat region; and

(v) a single-stranded nucleic acid comprising a nucleotide sequence of at least about 20 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 env open reading frame, an HIV-1 pol open reading frame or from an HIV-1 long terminal repeat region.

4. The composition of claim 1, wherein the duplex is bound to a solid support.

5. The composition of claim 1, wherein the single-stranded nucleic acid is randomly generated from an HIV-1 nucleic acid.

6. The composition of claim 1, wherein the single-stranded nucleic acid comprises DNA.

7. The composition of claim 1, wherein the single-stranded nucleic acid comprises RNA.

8. The composition of claim 1, wherein single-stranded nucleic acid is a cDNA.

9. The composition of claim 1, wherein the label is attached to the single-stranded nucleic acid and wherein the label is not an additional nucleic acid.

10. The composition of claim 1, wherein the single-stranded nucleic acid comprises a non-HIV-1 nucleotide sequence.

11. The composition of claim 1, wherein the single-stranded nucleic acid is chemically made at least in part.

12. The composition of claim 1, wherein the HIV-1 nucleic acid is a full-length HIV-1 nucleic acid.

13. The composition of claim 1, wherein the HIV-1 nucleic acid is not a full-length HIV-1 nucleic acid.

14. A method for preparing a DNA construct specific for Human Immunodeficiency Virus Type-1 (HIV-1) comprising the step of inserting into a vector a nucleic acid of at least 18 contiguous nucleotides comprising a nucleotide sequence selected from the group consisting of

- (i) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₂ having ATCC Accession No. 40231;
 - (ii) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₃ having ATCC Accession No. 40232;
 - (iii) a nucleotide sequence of either strand of viral DNA from clone BH10 having ATCC Accession No. 40125;
 - (iv) a nucleotide sequence of either strand of viral DNA from clone BH5 having ATCC Accession No. 40126;
 - (v) a nucleotide sequence of either strand of viral DNA from clone BH8 having ATCC Accession No. 40127;
 - (vi) a nucleotide sequence of either strand of viral DNA from clone pHXB3 having ATCC Accession No. 67081;
 - (vii) a nucleotide sequence of either strand of viral DNA from clone pHXB-2D having ATCC Accession No. 67082;
 - (viii) a nucleotide sequence of either strand of viral DNA from *E. coli* clone X10-1 having ATCC Accession No. 67083; and
 - (ix) an HIV-1 nucleotide sequence from the H9/HTLV-III cell line having ATCC Accession No. CRL 8543;
- whereby a DNA construct comprising an inserted nucleic acid is obtained.

15. The method according to claim 14, wherein the DNA construct permits making an RNA transcript of the inserted nucleic acid.

16. A method for replicating DNA specific for HIV-1 comprising the step of growing a cell containing the DNA construct of claim **14** under conditions whereby the inserted nucleic acid is replicated.

17. A method for producing a recombinant HIV-1 polypeptide comprising the step of growing a cell containing the DNA construct of claim **14** under conditions whereby the inserted nucleic acid is expressed to allow production of the recombinant HIV-1 polypeptide in the cell.

18. A nucleic acid of at least 18 contiguous nucleotides comprising a nucleotide sequence selected from the group consisting of

- (i) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₂ having ATCC Accession No. 40231;
- (ii) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₃ having ATCC Accession No. 40232;
- (iii) a nucleotide sequence of either strand of viral DNA from clone BH10 having ATCC Accession No. 40125;
- (iv) a nucleotide sequence of either strand of viral DNA from clone BH5 having ATCC Accession No. 40126;
- (v) a nucleotide sequence of either strand of viral DNA from clone BH8 having ATCC Accession No. 40127;
- (vi) a nucleotide sequence of either strand of viral DNA from clone pHXB3 having ATCC Accession No. 67081;
- (vii) a nucleotide sequence of either strand of viral DNA from clone pHXB-2D having ATCC Accession No. 67082;
- (viii) a nucleotide sequence of either strand of viral DNA from *E. coli* clone X10-1 having ATCC Accession No. 67083; and
- (ix) a Human Immunodeficiency Virus Type-1 (HIV-1) nucleotide sequence from the H9/HTLV-III cell line having ATCC Accession No. CRL 8543;

wherein the nucleic acid does not form a duplex with HTLV-I and HTLV-II nucleic acids under conditions of stringency for hybridization under which the nucleic acid forms a duplex with an HIV-1 nucleic acid; and wherein the nucleic acid is covalently attached to a solid support.

19. The nucleic acid of claim **18**, wherein the nucleic acid comprises a nucleotide sequence which is part of an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or part of an HIV-1 long terminal repeat region.

20. The nucleic acid of claim **18**, wherein the nucleic acid is selected from the group consisting of

- (i) a nucleic acid consisting of from 18 to 103 nucleotides comprising a nucleotide sequence of at least 18 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;
- (ii) a nucleic acid consisting of from 32 to 103 nucleotides comprising a sequence of at least 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;
- (iii) a nucleic acid consisting of from 20 to 100 nucleotides comprising a sequence of at least 20 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;
- (iv) a nucleic acid comprising a sequence of at least about 32 contiguous nucleotides from an HIV-1 gag open read-

ing frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region; and

- (v) a nucleic acid comprising a sequence of at least about 20 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region.

21. The nucleic acid of claim **18**, wherein the nucleic acid is a restriction fragment from an HIV-1 nucleic acid.

22. The nucleic acid of claim **18**, wherein the nucleic acid is randomly generated from an HIV-1 nucleic acid.

23. The nucleic acid of claim **18**, wherein the nucleic acid comprises DNA.

24. The nucleic acid of claim **18**, wherein the nucleic acid comprises RNA.

25. The nucleic acid of claim **18**, wherein the nucleic acid is a cDNA.

26. The nucleic acid of claim **18**, wherein the nucleic acid comprises a label.

27. The nucleic acid of claim **18**, wherein the nucleic acid comprises a non-HIV-1 nucleotide sequence.

28. The nucleic acid of claim **18**, wherein the nucleic acid is chemically made at least in part.

29. The nucleic acid of claim **18**, wherein the nucleic acid is a double-stranded nucleic acid.

30. The nucleic acid of claim **18**, wherein the nucleic acid is a single-stranded nucleic acid.

31. A single-stranded nucleic acid of at least 18 contiguous nucleotides comprising a nucleotide sequence selected from the group consisting of

- (i) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₂ having ATCC Accession No. 40231;
- (ii) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₃ having ATCC Accession No. 40232;
- (iii) a nucleotide sequence of either strand of viral DNA from clone BH10 having ATCC Accession No. 40125;
- (iv) a nucleotide sequence of either strand of viral DNA from clone BH5 having ATCC Accession No. 40126;
- (v) a nucleotide sequence of either strand of viral DNA from clone BH8 having ATCC Accession No. 40127;
- (vi) a nucleotide sequence of either strand of viral DNA from clone pHXB3 having ATCC Accession No. 67081;
- (vii) a nucleotide sequence of either strand of viral DNA from clone pHXB-2D having ATCC Accession No. 67082;
- (viii) a nucleotide sequence of either strand of viral DNA from *E. coli* clone X10-1 having ATCC Accession No. 67083; and
- (ix) a Human Immunodeficiency Virus Type-1 (HIV-1) nucleotide sequence from the H9/HTLV-III cell line having ATCC Accession No. CRL 8543;

wherein the single-stranded nucleic acid does not form a duplex with HTLV-I and HTLV-II nucleic acids under conditions of stringency for hybridization under which the single-stranded nucleic acid forms a duplex with an HIV-1 nucleic acid;

wherein the single-stranded nucleic acid is within a duplex comprising the HIV-1 nucleic acid; and

wherein the duplex is covalently attached to a solid support.

32. The single-stranded nucleic acid of claim **31**, wherein the single-stranded nucleic acid is complementary to a sequence which is part of an HIV-1 gag open reading frame,

an HIV-1 pol open reading frame, an HIV-1 env open reading frame or part of an HIV-1 long terminal repeat region.

33. The single-stranded nucleic acid of claim **31**, wherein the single-stranded nucleic acid is selected from the group consisting of

(i) a single-stranded nucleic acid consisting of from 18 to 103 nucleotides comprising a sequence of at least 18 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;

(ii) a single-stranded nucleic acid consisting of from 32 to 103 nucleotides comprising a sequence of at least 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;

(iii) a single-stranded nucleic acid consisting of from 20 to 100 nucleotides comprising a sequence of at least 20 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;

(iv) a single-stranded nucleic acid comprising a sequence of at least about 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region; and

(v) a single-stranded nucleic acid comprising a sequence of at least about 20 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region.

34. The single-stranded nucleic acid of claim **31**, wherein the HIV-1 nucleic acid is bound to a solid support.

35. The single-stranded nucleic acid of claim **31**, wherein the single-stranded nucleic acid is randomly generated from an HIV-1 nucleic acid.

36. The single-stranded nucleic acid of claim **31**, wherein the single-stranded nucleic acid comprises DNA.

37. The single-stranded nucleic acid of claim **31**, wherein the single-stranded nucleic acid comprises RNA.

38. The single-stranded nucleic acid of claim **31**, wherein the single-stranded nucleic acid is a cDNA.

39. The single-stranded nucleic acid of claim **31**, wherein the single-stranded nucleic acid comprises a label.

40. The single-stranded nucleic acid of claim **31**, wherein the single-stranded nucleic acid comprises a non-HIV-1 nucleotide sequence.

41. The single-stranded nucleic acid of claim **31**, wherein the single-stranded nucleic acid is chemically made at least in part.

42. The single-stranded nucleic acid of claim **31**, wherein the HIV-1 nucleic acid is a full-length HIV-1 nucleic acid.

43. The single-stranded nucleic acid of claim **31**, wherein the HIV-1 nucleic acid is not a full-length HIV-1 nucleic acid.

44. A nucleic acid of at least 18 contiguous nucleotides comprising a nucleotide sequence selected from the group consisting of

(i) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₂ having ATCC Accession No. 40231;

(ii) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₃ having ATCC Accession No. 40232;

(iii) a nucleotide sequence of either strand of viral DNA from clone BH10 having ATCC Accession No. 40125;

(iv) a nucleotide sequence of either strand of viral DNA from clone BH5 having ATCC Accession No. 40126;

(v) a nucleotide sequence of either strand of viral DNA from clone BH8 having ATCC Accession No. 40127;

(vi) a nucleotide sequence of either strand of viral DNA from clone pHXB3 having ATCC Accession No. 67081;

(vii) a nucleotide sequence of either strand of viral DNA from clone pHXB-2D having ATCC Accession No. 67082;

(viii) a nucleotide sequence of either strand of viral DNA from *E. coli* clone X10-1 having ATCC Accession No. 67083; and

(ix) a Human Immunodeficiency Virus Type-1 (HIV-1) nucleotide sequence from the H9/HTLV-III cell line having ATCC Accession No. CRL 8543;

wherein the nucleic acid does not form a duplex with HTLV-I and HTLV-II nucleic acids under conditions of stringency for hybridization under which the nucleic acid forms a duplex with an HIV-1 nucleic acid;

wherein the nucleic acid comprises a detectable label covalently attached to the nucleic acid; and

wherein the detectable label is not an additional nucleic acid.

45. The nucleic acid of claim **44**, wherein the nucleic acid comprises a nucleotide sequence which is part of an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or part of an HIV-1 long terminal repeat region.

46. The nucleic acid of claim **44**, wherein the nucleic acid is selected from the group consisting of

(i) a nucleic acid consisting of from 18 to 103 nucleotides comprising a sequence of at least 18 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;

(ii) a nucleic acid consisting of from 32 to 103 nucleotides comprising a sequence of at least 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;

(iii) a nucleic acid consisting of from 20 to 100 nucleotides comprising a sequence of at least 20 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;

(iv) a nucleic acid comprising a sequence of at least about 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region; and

(v) a nucleic acid comprising a sequence of at least about 20 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region.

47. The nucleic acid of claim **44**, wherein the nucleic acid is a restriction fragment from an HIV-1 nucleic acid.

48. The nucleic acid of claim **44**, wherein the nucleic acid is randomly generated from an HIV-1 nucleic acid.

49. The nucleic acid of claim **44**, wherein the nucleic acid comprises DNA.

50. The nucleic acid of claim **44**, wherein the nucleic acid comprises RNA.

51. The nucleic acid of claim **44**, wherein the nucleic acid is a cDNA.

52. The nucleic acid of claim **44**, wherein the nucleic acid comprises a non-HIV-1 nucleotide sequence.

53. The nucleic acid of claim **44**, wherein the nucleic acid is chemically made at least in part.

54. The nucleic acid of claim **44**, wherein the nucleic acid is a double-stranded nucleic acid.

55. The nucleic acid of claim **44**, wherein the nucleic acid is a single-stranded nucleic acid.

56. A nucleic acid of at least 18 contiguous nucleotides comprising a nucleotide sequence selected from the group consisting of

- (i) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₂ having ATCC Accession No. 40231;
- (ii) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₃ having ATCC Accession No. 40232;
- (iii) a nucleotide sequence of either strand of viral DNA from clone BH10 having ATCC Accession No. 40125;
- (iv) a nucleotide sequence of either strand of viral DNA from clone BH5 having ATCC Accession No. 40126;
- (v) a nucleotide sequence of either strand of viral DNA from clone BH8 having ATCC Accession No. 40127;
- (vi) a nucleotide sequence of either strand of viral DNA from clone pHXB3 having ATCC Accession No. 67081;
- (vii) a nucleotide sequence of either strand of viral DNA from clone pHXB-2D having ATCC Accession No. 67082;
- (viii) a nucleotide sequence of either strand of viral DNA from *E. coli* clone X10-1 having ATCC Accession No. 67083; and

(ix) a Human Immunodeficiency Virus Type-1 (HIV-1) nucleotide sequence from the H9/HTLV-III cell line having ATCC Accession No. CRL 8543;

wherein the nucleic acid does not form a duplex with HTLV-I and HTLV-II nucleic acids under conditions of stringency for hybridization under which the nucleic acid forms a duplex with an HIV-1 nucleic acid;

wherein the nucleic acid is outside of a mammalian cell and outside of a viral particle; and

wherein the nucleic acid is attached to a non-HIV-1 nucleic acid through a covalent bond.

57. The nucleic acid of claim **56**, wherein the nucleic acid is complementary to a sequence which is part of an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or part of an HIV-1 long terminal repeat region.

58. The nucleic acid of claim **56**, wherein the nucleic acid is selected from the group consisting of

- (i) a nucleic acid consisting of from 18 to 103 nucleotides comprising a sequence of at least 18 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;
- (ii) a nucleic acid consisting of from 32 to 103 nucleotides comprising a sequence of at least 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;
- (iii) a nucleic acid consisting of from 20 to 100 nucleotides comprising a sequence of at least 20 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;
- (iv) a nucleic acid comprising a sequence of at least about 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region; and

(v) a nucleic acid comprising a sequence of at least about 20 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region.

59. The nucleic acid of claim **56**, wherein the nucleic acid is a restriction fragment from an HIV-1 nucleic acid.

60. The nucleic acid of claim **56**, wherein the nucleic acid is randomly generated from an HIV-1 nucleic acid.

61. The nucleic acid of claim **56**, wherein the nucleic acid comprises DNA.

62. The nucleic acid of claim **56**, wherein the nucleic acid comprises RNA.

63. The nucleic acid of claim **56**, wherein the nucleic acid is a cDNA.

64. The nucleic acid of claim **56**, wherein the nucleic acid comprises a label.

65. The nucleic acid of claim **56**, wherein the nucleic acid is chemically made at least in part.

66. The nucleic acid of claim **56**, wherein the nucleic acid is a double-stranded nucleic acid.

67. The nucleic acid of claim **56**, wherein the nucleic acid is a single-stranded nucleic acid.

68. A composition comprising a duplex formed between:
(A) a single-stranded nucleic acid of between 18 and 103 contiguous nucleotides and comprising a nucleotide sequence selected from the group consisting of:

- (i) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₂ having ATCC Accession No. 40231;
- (ii) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₃ having ATCC Accession No. 40232;
- (iii) a nucleotide sequence of either strand of viral DNA from clone BH10 having ATCC Accession No. 40125;
- (iv) a nucleotide sequence of either strand of viral DNA from clone BH5 having ATCC Accession No. 40126;
- (v) a nucleotide sequence of either strand of viral DNA from clone BH8 having ATCC Accession No. 40127;
- (vi) a nucleotide sequence of either strand of viral DNA from clone pHXB3 having ATCC Accession No. 67081;
- (vii) a nucleotide sequence of either strand of viral DNA from clone pHXB-2D having ATCC Accession No. 67082;
- (viii) a nucleotide sequence of either strand of viral DNA from *E. coli* clone X10-1 having ATCC Accession No. 67083; and
- (ix) a Human Immunodeficiency Virus Type-1 (HIV-1) nucleotide sequence from the H9/HTLV-III cell line having ATCC Accession No. CRL 8543; and

(B) an HIV-1 nucleic acid selected from the group consisting of:

- (a) an HIV-1 nucleic acid comprising a nucleotide sequence encoding a full-length gag polypeptide or its complement;
- (b) an HIV-1 nucleic acid comprising a nucleotide sequence encoding a full-length pol polypeptide or its complement;
- (c) an HIV-1 nucleic acid comprising a nucleotide sequence encoding a full-length env polypeptide or its complement; and
- (d) an HIV-1 nucleic acid comprising a nucleotide sequence for a long terminal repeat region comprising R and U₃ regions or their complements;

wherein the single-stranded nucleic acid of (A) does not form a duplex with HTLV-I and HTLV-II nucleic acids

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under conditions of stringency for hybridization under which the nucleic acid of (A) forms a duplex with the HIV-1 nucleic acid of (B);
 wherein the duplex is outside of a mammalian cell and outside of a viral particle;
 wherein the duplex comprises a double-stranded region of between 18 and 103 contiguous nucleotides and a single-stranded region on either side of the double-stranded region that is longer than the double-stranded region; and
 wherein the single-stranded nucleic acid comprises a label.

69. The composition of claim **68**, wherein the single-stranded nucleic acid comprises a nucleotide sequence which is part of an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or part of an HIV-1 long terminal repeat region.

70. The composition of claim **68**, wherein the single-stranded nucleic acid is selected from the group consisting of

- (i) a single-stranded nucleic acid consisting of from 18 to 103 nucleotides comprising a sequence of at least 18 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;
- (ii) a single-stranded nucleic acid consisting of from 32 to 103 nucleotides comprising a sequence of at least 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;
- (iii) a single-stranded nucleic acid consisting of from 20 to 100 nucleotides comprising a sequence of at least 20 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;
- (iv) a single-stranded nucleic acid comprising a sequence of at least about 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region; and
- (v) a single-stranded nucleic acid comprising a sequence of at least about 20 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region.

71. The composition of claim **68**, wherein the single-stranded nucleic acid is randomly generated from an HIV-1 nucleic acid.

72. The composition of claim **68**, wherein the single-stranded nucleic acid comprises DNA.

73. The composition of claim **68**, wherein the single-stranded nucleic acid comprises RNA.

74. The composition of claim **68**, wherein the single-stranded nucleic acid is a cDNA.

75. The composition of claim **68**, wherein the label is attached to the single-stranded nucleic acid and wherein the label is not an additional nucleic acid.

76. The composition of claim **68**, wherein the single-stranded nucleic acid comprises a non-HIV-1 nucleotide sequence.

77. The composition of claim **68**, wherein the single-stranded nucleic acid is chemically made at least in part.

78. The composition of claim **68**, wherein the single-stranded nucleic acid is bound to a solid support.

79. The composition of claim **68**, wherein the duplex is bound to a solid support.

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80. The composition of claim **68**, wherein the HIV-1 nucleic acid is a full-length HIV-1 nucleic acid.

81. The composition of claim **68**, wherein the HIV-1 nucleic acid is not a full-length HIV-1 nucleic acid.

82. A composition comprising:

(A) a duplex; and

(B) a compound selected from the group consisting of sodium saline citrate, formamide, and dextran sulfate; wherein the duplex comprises a Human Immunodeficiency Virus Type-1 (HIV-1) nucleic acid having a length of at least 300 nucleotides hybridized to a single-stranded nucleic acid of between 18 and 103 contiguous nucleotides and comprising a nucleotide sequence selected from the group consisting of:

- (i) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₂ having ATCC Accession No. 40231;
- (ii) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₃ having ATCC Accession No. 40232;
- (iii) a nucleotide sequence of either strand of viral DNA from clone BH10 having ATCC Accession No. 40125;
- (iv) a nucleotide sequence of either strand of viral DNA from clone BH5 having ATCC Accession No. 40126;
- (v) a nucleotide sequence of either strand of viral DNA from clone BH8 having ATCC Accession No. 40127;
- (vi) a nucleotide sequence of either strand of viral DNA from clone pHXB3 having ATCC Accession No. 67081;
- (vii) a nucleotide sequence of either strand of viral DNA from clone pHXB-2D having ATCC Accession No. 67082;
- (viii) a nucleotide sequence of either strand of viral DNA from *E. coli* clone X10-1 having ATCC Accession No. 67083; and
- (ix) a Human Immunodeficiency Virus Type-1 (HIV-1) nucleotide sequence from the H9/HTLV-III cell line having ATCC Accession No. CRL 8543;

wherein the single-stranded nucleic acid does not form a duplex with HTLV-I and HTLV-II nucleic acids under conditions of stringency for hybridization under which the single-stranded nucleic acid forms a duplex with the HIV-1 nucleic acid; and

wherein the duplex comprises a double-stranded region and a single-stranded region that is longer than the double-stranded region.

83. The composition of claim **82**, wherein the single-stranded nucleic acid comprises a sequence which is part of an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or part of an HIV-1 long terminal repeat region.

84. The composition of claim **82**, wherein the single-stranded nucleic acid is selected from the group consisting of

- (i) a single-stranded nucleic acid consisting of from 18 to 103 nucleotides comprising a sequence of at least 18 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;
- (ii) a single-stranded nucleic acid consisting of from 32 to 103 nucleotides comprising a sequence of at least 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;
- (iii) a single-stranded nucleic acid consisting of from 20 to 100 nucleotides comprising a sequence of at least 20 contiguous nucleotides from an HIV-1 gag open reading

frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;

- (iv) a single-stranded nucleic acid comprising a sequence of at least about 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region; and
- (v) a single-stranded nucleic acid comprising a sequence of at least about 20 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region.

85. The composition of claim **82**, wherein the single-stranded nucleic acid is randomly generated from an HIV-1 nucleic acid.

86. The composition of claim **82**, wherein the single-stranded nucleic acid comprises DNA.

87. The composition of claim **82**, wherein the single-stranded nucleic acid comprises RNA.

88. The composition of claim **82**, wherein the single-stranded nucleic acid is a cDNA.

89. The composition of claim **82**, wherein the single-stranded nucleic acid comprises a label.

90. The composition of claim **82**, wherein the single-stranded nucleic acid comprises a non-HIV-1 nucleotide sequence.

91. The composition of claim **82**, wherein the single-stranded nucleic acid is chemically made at least in part.

92. The composition of claim **82**, wherein the single-stranded nucleic acid is bound to a solid support.

93. The composition of claim **82**, wherein the duplex is bound to a solid support.

94. The composition of claim **82**, wherein the HIV-1 nucleic acid is a full-length HIV-1 nucleic acid.

95. The composition of claim **82**, wherein the HIV-1 nucleic acid is not a full-length HIV-1 nucleic acid.

96. A composition comprising a duplex formed between a Human Immunodeficiency Virus Type-1 (HIV-1) nucleic acid and a single-stranded nucleic acid of at least 18 contiguous nucleotides and comprising a nucleotide sequence selected from the group consisting of:

- (i) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₂ having ATCC Accession No. 40231;
- (ii) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₃ having ATCC Accession No. 40232;
- (iii) a nucleotide sequence of either strand of viral DNA from clone BH10 having ATCC Accession No. 40125;
- (iv) a nucleotide sequence of either strand of viral DNA from clone BH5 having ATCC Accession No. 40126;
- (v) a nucleotide sequence of either strand of viral DNA from clone BH8 having ATCC Accession No. 40127;
- (vi) a nucleotide sequence of either strand of viral DNA from clone pHXB3 having ATCC Accession No. 67081;
- (vii) a nucleotide sequence of either strand of viral DNA from clone pHXB-2D having ATCC Accession No. 67082;
- (viii) a nucleotide sequence of either strand of viral DNA from *E. coli* clone X10-1 having ATCC Accession No. 67083; and
- (ix) an HIV-1 nucleotide sequence from the H9/HTLV-III cell line having ATCC Accession No. CRL 8543; wherein the single-stranded nucleic acid does not form a duplex with HTLV-I and HTLV-II nucleic acids under conditions of stringency for hybridization under

which the single-stranded nucleic acid forms a duplex with the HIV-1 nucleic acid;

wherein the single-stranded nucleic acid consists of DNA;

wherein the duplex is outside of a mammalian cell;

wherein the duplex comprises a double-stranded region of at least 18 contiguous nucleotides and a single-stranded region on either side of the double-stranded region that is longer than the double-stranded region; and

wherein the duplex is bound to a solid support.

97. The composition of claim **96**, wherein the single-stranded nucleic acid comprises a nucleotide sequence which is part of an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or part of an HIV-1 long terminal repeat region.

98. The composition of claim **96**, wherein the single-stranded nucleic acid is selected from the group consisting of:

- (i) a single-stranded nucleic acid consisting of from 18 to 103 nucleotides comprising a nucleotide sequence of at least 18 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 env open reading frame, an HIV-1 pol open reading frame or from an HIV-1 long terminal repeat region;
- (ii) a single-stranded nucleic acid consisting of from 32 to 103 nucleotides comprising a nucleotide sequence of at least 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 env open reading frame, an HIV-1 pol open reading frame or from an HIV-1 long terminal repeat region;
- (iii) a single-stranded nucleic acid consisting of from 20 to 100 nucleotides comprising a nucleotide sequence of at least 20 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 env open reading frame, an HIV-1 pol open reading frame or from an HIV-1 long terminal repeat region;
- (iv) a single-stranded nucleic acid comprising a nucleotide sequence of at least about 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 env open reading frame, an HIV-1 pol open reading frame or from an HIV-1 long terminal repeat region; and
- (v) a single-stranded nucleic acid comprising a nucleotide sequence of at least about 20 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 env open reading frame, an HIV-1 pol open reading frame or from an HIV-1 long terminal repeat region.

99. The composition of claim **96**, further comprising a compound selected from the group consisting of sodium saline citrate, formamide, and dextran sulfate.

100. The composition of claim **96**, wherein the single-stranded nucleic acid is randomly generated from an HIV-1 nucleic acid.

101. The composition of claim **96**, wherein the single-stranded nucleic acid comprises a label.

102. The composition of claim **96**, wherein the single-stranded nucleic acid comprises a non-HIV-1 DNA nucleotide sequence.

103. The composition of claim **96**, wherein the single-stranded nucleic acid is chemically made at least in part.

104. The composition of claim **96**, wherein the HIV-1 nucleic acid is a full-length HIV-1 nucleic acid.

105. The composition of claim **96**, wherein the HIV-1 nucleic acid is not a full-length HIV-1 nucleic acid.

106. A single-stranded nucleic acid of at least 18 contiguous nucleotides comprising a nucleotide sequence selected from the group consisting of:

- (i) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₂ having ATCC Accession No. 40231;
- (ii) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₃ having ATCC Accession No. 40232;
- (iii) a nucleotide sequence of either strand of viral DNA from clone BH10 having ATCC Accession No. 40125;
- (iv) a nucleotide sequence of either strand of viral DNA from clone BH5 having ATCC Accession No. 40126;
- (v) a nucleotide sequence of either strand of viral DNA from clone BH8 having ATCC Accession No. 40127;
- (vi) a nucleotide sequence of either strand of viral DNA from clone pHXB3 having ATCC Accession No. 67081;
- (vii) a nucleotide sequence of either strand of viral DNA from clone pHXB-2D having ATCC Accession No. 67082;
- (viii) a nucleotide sequence of either strand of viral DNA from *E. coli* clone X10-1 having ATCC Accession No. 67083; and
- (ix) a Human Immunodeficiency Virus Type-1 (HIV-1) nucleotide sequence from the H9/HTLV-III cell line having ATCC Accession No. CRL 8543;

wherein the single-stranded nucleic acid does not form a duplex with HTLV-I and HTLV-II nucleic acids under conditions of stringency for hybridization under which the single-stranded nucleic acid forms a duplex with an HIV-1 nucleic acid;

wherein the single-stranded nucleic acid is outside of a mammalian cell and outside of a viral particle;

wherein the single-stranded nucleic acid consists of DNA; and

wherein the single-stranded nucleic acid is attached to a non-HIV-1 DNA nucleic acid through a covalent bond.

107. The single-stranded nucleic acid of claim **106**, wherein the nucleic acid is complementary to a sequence which is part of an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or part of an HIV-1 long terminal repeat region.

108. The single-stranded nucleic acid of claim **106**, wherein the single-stranded nucleic acid is selected from the group consisting of

- (i) a single-stranded nucleic acid consisting of from 18 to 103 nucleotides comprising a sequence of at least 18 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;
- (ii) a single-stranded nucleic acid consisting of from 32 to 103 nucleotides comprising a sequence of at least 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;
- (iii) a single-stranded nucleic acid consisting of from 20 to 100 nucleotides comprising a sequence of at least 20 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;
- (iv) a single-stranded nucleic acid comprising a sequence of at least about 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region; and
- (v) a single-stranded nucleic acid comprising a sequence of at least about 20 contiguous nucleotides from an HIV-1

gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region.

109. The single-stranded nucleic acid of claim **106**, wherein the single-stranded nucleic acid is randomly generated from an HIV-1 nucleic acid.

110. The single-stranded nucleic acid of claim **106**, wherein the single-stranded nucleic acid comprises a label.

111. The single-stranded nucleic acid of claim **106**, wherein the single-stranded nucleic acid is chemically made at least in part.

112. A composition comprising a duplex formed between:

(A) a single-stranded nucleic acid of between 18 and 103 contiguous nucleotides and comprising a nucleotide sequence selected from the group consisting of:

- (i) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₂ having ATCC Accession No. 40231;
- (ii) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₃ having ATCC Accession No. 40232;
- (iii) a nucleotide sequence of either strand of viral DNA from clone BH10 having ATCC Accession No. 40125;
- (iv) a nucleotide sequence of either strand of viral DNA from clone BH5 having ATCC Accession No. 40126;
- (v) a nucleotide sequence of either strand of viral DNA from clone BH8 having ATCC Accession No. 40127;
- (vi) a nucleotide sequence of either strand of viral DNA from clone pHXB3 having ATCC Accession No. 67081;
- (vii) a nucleotide sequence of either strand of viral DNA from clone pHXB-2D having ATCC Accession No. 67082;
- (viii) a nucleotide sequence of either strand of viral DNA from *E. coli* clone X10-1 having ATCC Accession No. 67083; and
- (ix) a Human Immunodeficiency Virus Type-1 (HIV-1) nucleotide sequence from the H9/HTLV-III cell line having ATCC Accession No. CRL 8543; and

(B) an HIV-1 nucleic acid selected from the group consisting of:

- (a) an HIV-1 nucleic acid comprising a nucleotide sequence encoding a full-length gag polypeptide or its complement;
- (b) an HIV-1 nucleic acid comprising a nucleotide sequence encoding a full-length pol polypeptide or its complement;
- (c) an HIV-1 nucleic acid comprising a nucleotide sequence encoding a full-length env polypeptide or its complement; and
- (d) an HIV-1 nucleic acid comprising a nucleotide sequence for a long terminal repeat region comprising R and U₃ regions or their complements;

wherein the single-stranded nucleic acid of (A) does not form a duplex with HTLV-I and HTLV-II nucleic acids under conditions of stringency for hybridization under which the single-stranded nucleic acid of (A) forms a duplex with the HIV-1 nucleic acid of (B);

wherein the single-stranded nucleic acid of (A) consists of DNA;

wherein the duplex is outside of a mammalian cell and outside of a viral particle;

wherein the duplex comprises a double-stranded region of between 18 and 103 contiguous nucleotides and a single-stranded region on either side of the double-stranded region that is longer than the double-stranded region; and

wherein either the single-stranded nucleic acid or the duplex is bound to a solid support.

113. The composition of claim **112**, wherein the single-stranded nucleic acid comprises a nucleotide sequence which is part of an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or part of an HIV-1 long terminal repeat region.

114. The composition of claim **112**, wherein the single-stranded nucleic acid is selected from the group consisting of

- (i) a single-stranded nucleic acid consisting of from 18 to 103 nucleotides comprising a sequence of at least 18 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;
- (ii) a single-stranded nucleic acid consisting of from 32 to 103 nucleotides comprising a sequence of at least 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;
- (iii) a single-stranded nucleic acid consisting of from 20 to 100 nucleotides comprising a sequence of at least 20 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;
- (iv) a single-stranded nucleic acid comprising a sequence of at least about 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region; and
- (v) a single-stranded nucleic acid comprising a sequence of at least about 20 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region.

115. The composition of claim **112**, wherein the single-stranded nucleic acid is randomly generated from an HIV-1 nucleic acid.

116. The composition of claim **112**, wherein the single-stranded nucleic acid comprises a label.

117. The composition of claim **112**, wherein the single-stranded nucleic acid comprises a non-HIV-1 DNA nucleotide sequence.

118. The composition of claim **112**, wherein the single-stranded nucleic acid is chemically made at least in part.

119. The composition of claim **112**, wherein the single-stranded nucleic acid is bound to the solid support.

120. The composition of claim **112**, wherein the duplex is bound to the solid support.

121. The composition of claim **112**, wherein the HIV-1 nucleic acid is a full-length HIV-1 nucleic acid.

122. The composition of claim **112**, wherein the HIV-1 nucleic acid is not a full-length HIV-1 nucleic acid.

123. A composition comprising:

(A) a single-stranded nucleic acid of between 18 and 103 contiguous nucleotides and comprising a nucleotide sequence selected from the group consisting of:

- (i) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₂ having ATCC Accession No. 40231;
- (ii) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₃ having ATCC Accession No. 40232;
- (iii) a nucleotide sequence of either strand of viral DNA from clone BH10 having ATCC Accession No. 40125;

(iv) a nucleotide sequence of either strand of viral DNA from clone BH5 having ATCC Accession No. 40126;

(v) a nucleotide sequence of either strand of viral DNA from clone BH8 having ATCC Accession No. 40127;

(vi) a nucleotide sequence of either strand of viral DNA from clone pHXB3 having ATCC Accession No. 67081;

(vii) a nucleotide sequence of either strand of viral DNA from clone pHXB-2D having ATCC Accession No. 67082;

(viii) a nucleotide sequence of either strand of viral DNA from *E. coli* clone X10-1 having ATCC Accession No. 67083; and

(ix) a Human Immunodeficiency Virus Type-1 (HIV-1) nucleotide sequence from the H9/HTLV-III cell line having ATCC Accession No. CRL 8543; and

(B) a compound selected from the group consisting of sodium saline citrate, formamide, and dextran sulfate; wherein the single-stranded nucleic acid does not form a duplex with HTLV-I and HTLV-II nucleic acids under conditions of stringency for hybridization under which the single-stranded nucleic acid forms a duplex with an HIV-1 nucleic acid.

124. The composition of claim **123**, wherein the single-stranded nucleic acid comprises a sequence which is part of an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or part of an HIV-1 long terminal repeat region.

125. The composition of claim **123**, wherein the single-stranded nucleic acid is selected from the group consisting of:

(i) a single-stranded nucleic acid consisting of from 18 to 103 nucleotides comprising a sequence of at least 18 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;

(ii) a single-stranded nucleic acid consisting of from 32 to 103 nucleotides comprising a sequence of at least 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;

(iii) a single-stranded nucleic acid consisting of from 20 to 100 nucleotides comprising a sequence of at least 20 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region;

(iv) a single-stranded nucleic acid comprising a sequence of at least about 32 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region; and

(v) a single-stranded nucleic acid comprising a sequence of at least about 20 contiguous nucleotides from an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or from an HIV-1 long terminal repeat region.

126. The composition of claim **123**, wherein the single-stranded nucleic acid is randomly generated from an HIV-1 nucleic acid.

127. The composition of claim **123**, wherein the single-stranded nucleic acid comprises DNA.

128. The composition of claim **123**, wherein the single-stranded nucleic acid comprises RNA.

129. The composition of claim **123**, wherein the single-stranded nucleic acid is a cDNA.

130. The composition of claim **123**, wherein the single-stranded nucleic acid comprises a label.

131. The composition of claim **123**, wherein the single-stranded nucleic acid comprises a non-HIV-1 nucleotide sequence.

132. The composition of claim **123**, wherein the single-stranded nucleic acid is chemically made at least in part.

133. The composition of claim **123**, wherein the single-stranded nucleic acid is bound to a solid support.

134. A composition comprising a duplex formed between a Human Immunodeficiency Virus Type-1 (HIV-1) nucleic acid and a single-stranded nucleic acid comprising a nucleotide sequence selected from the group consisting of:

- (i) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₂ having ATCC Accession No. 40231;
- (ii) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₃ having ATCC Accession No. 40232;
- (iii) a nucleotide sequence of either strand of viral DNA from clone BH10 having ATCC Accession No. 40125;
- (iv) a nucleotide sequence of either strand of viral DNA from clone BH5 having ATCC Accession No. 40126;
- (v) a nucleotide sequence of either strand of viral DNA from clone BH8 having ATCC Accession No. 40127;
- (vi) a nucleotide sequence of either strand of viral DNA from clone pHXB3 having ATCC Accession No. 67081;
- (vii) a nucleotide sequence of either strand of viral DNA from clone pHXB-2D having ATCC Accession No. 67082;
- (viii) a nucleotide sequence of either strand of viral DNA from *E. coli* clone X10-1 having ATCC Accession No. 67083; and
- (ix) an HIV-1 nucleotide sequence from the H9/HTLV-III cell line having ATCC Accession No. CRL 8543; wherein the single-stranded nucleic acid does not form a duplex with HTLV-I and HTLV-II nucleic acids under conditions of stringency for hybridization under which the single-stranded nucleic acid forms a duplex with the HIV-1 nucleic acid; wherein the duplex is outside of a mammalian cell and outside of an HIV-1 particle; wherein the duplex comprises a double-stranded region and a single-stranded region on either side of the double-stranded region that is longer than the double-stranded region; and wherein the single-stranded nucleic acid comprises a label.

135. The composition of claim **134**, wherein the single-stranded nucleic acid comprises a nucleotide sequence which is part of an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or part of an HIV-1 long terminal repeat region.

136. The composition of claim **134**, wherein the single-stranded nucleic acid comprises DNA.

137. The composition of claim **134**, wherein the single-stranded nucleic acid comprises RNA.

138. A composition comprising a duplex formed between a Human Immunodeficiency Virus Type-1 (HIV-1) nucleic acid and a single-stranded nucleic acid comprising a nucleotide sequence selected from the group consisting of:

- (i) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₂ having ATCC Accession No. 40231;
- (ii) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₃ having ATCC Accession No. 40232;

(iii) a nucleotide sequence of either strand of viral DNA from clone BH10 having ATCC Accession No. 40125;

(iv) a nucleotide sequence of either strand of viral DNA from clone BH5 having ATCC Accession No. 40126;

(v) a nucleotide sequence of either strand of viral DNA from clone BH8 having ATCC Accession No. 40127;

(vi) a nucleotide sequence of either strand of viral DNA from clone pHXB3 having ATCC Accession No. 67081;

(vii) a nucleotide sequence of either strand of viral DNA from clone pHXB-2D having ATCC Accession No. 67082;

(viii) a nucleotide sequence of either strand of viral DNA from *E. coli* clone X10-1 having ATCC Accession No. 67083; and

(ix) an HIV-1 nucleotide sequence from the H9/HTLV-III cell line having ATCC Accession No. CRL 8543;

wherein the single-stranded nucleic acid does not form a duplex with HTLV-I and HTLV-II nucleic acids under conditions of stringency for hybridization under which the single-stranded nucleic acid forms a duplex with the HIV-1 nucleic acid;

wherein the duplex is outside of a mammalian cell and outside of an HIV-1 particle;

wherein the duplex comprises a double-stranded region and a single-stranded region on either side of the double-stranded region that is longer than the double-stranded region; and

wherein either the duplex or the single-stranded nucleic acid is bound to a solid support.

139. The composition of claim **138**, wherein the single-stranded nucleic acid comprises a nucleotide sequence which is part of an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or part of an HIV-1 long terminal repeat region.

140. The composition of claim **138**, wherein the single-stranded nucleic acid comprises DNA.

141. The composition of claim **138**, wherein the single-stranded nucleic acid comprises RNA.

142. A composition comprising:

a compound selected from the group consisting of sodium saline citrate, formamide, and dextran sulfate; and a duplex formed between a Human Immunodeficiency Virus Type-1 (HIV-1) nucleic acid and a single-stranded nucleic acid comprising a nucleotide sequence selected from the group consisting of:

(i) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₂ having ATCC Accession No. 40231;

(ii) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₃ having ATCC Accession No. 40232;

(iii) a nucleotide sequence of either strand of viral DNA from clone BH10 having ATCC Accession No. 40125;

(iv) a nucleotide sequence of either strand of viral DNA from clone BH5 having ATCC Accession No. 40126;

(v) a nucleotide sequence of either strand of viral DNA from clone BH8 having ATCC Accession No. 40127;

(vi) a nucleotide sequence of either strand of viral DNA from clone pHXB3 having ATCC Accession No. 67081;

(vii) a nucleotide sequence of either strand of viral DNA from clone pHXB-2D having ATCC Accession No. 67082;

(viii) a nucleotide sequence of either strand of viral DNA from *E. coli* clone X10-1 having ATCC Accession No. 67083; and

(ix) an HIV-1 nucleotide sequence from the H9/HTLV-III cell line having ATCC Accession No. CRL 8543;

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wherein the single-stranded nucleic acid does not form a duplex with HTLV-I and HTLV-II nucleic acids under conditions of stringency for hybridization under which the single-stranded nucleic acid forms a duplex with the HIV-1 nucleic acid;

wherein the duplex is outside of a mammalian cell and outside of an HIV-1 particle;

wherein the duplex comprises a double-stranded region and a single-stranded region on either side of the double-stranded region that is longer than the double-stranded region.

143. The composition of claim **142**, wherein the single-stranded nucleic acid comprises a nucleotide sequence which is part of an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or part of an HIV-1 long terminal repeat region.

144. The composition of claim **142**, wherein the single-stranded nucleic acid comprises DNA.

145. The composition of claim **142**, wherein the single-stranded nucleic acid comprises RNA.

146. A composition comprising a duplex formed between a Human Immunodeficiency Virus Type-1 (HIV-1) nucleic acid and a single-stranded nucleic acid comprising a nucleotide sequence selected from the group consisting of:

(i) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₂ having ATCC Accession No. 40231;

(ii) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₃ having ATCC Accession No. 40232;

(iii) a nucleotide sequence of either strand of viral DNA from clone BH10 having ATCC Accession No. 40125;

(iv) a nucleotide sequence of either strand of viral DNA from clone BH5 having ATCC Accession No. 40126;

(v) a nucleotide sequence of either strand of viral DNA from clone BH8 having ATCC Accession No. 40127;

(vi) a nucleotide sequence of either strand of viral DNA from clone pHXB3 having ATCC Accession No. 67081;

(vii) a nucleotide sequence of either strand of viral DNA from clone pHXB-2D having ATCC Accession No. 67082;

(viii) a nucleotide sequence of either strand of viral DNA from *E. coli* clone X10-1 having ATCC Accession No. 67083; and

(ix) an HIV-1 nucleotide sequence from the H9/HTLV-III cell line having ATCC Accession No. CRL 8543;

wherein the single-stranded nucleic acid does not form a duplex with HTLV-I and HTLV-II nucleic acids under conditions of stringency for hybridization under which the single-stranded nucleic acid forms a duplex with the HIV-1 nucleic acid;

wherein the duplex is outside of a mammalian cell and outside of an HIV-1 particle;

wherein the duplex comprises a double-stranded region and a single-stranded region on either side of the double-stranded region that is longer than the double-stranded region; and

wherein the single-stranded nucleic acid is attached to a non-HIV-1 nucleic acid through a covalent bond.

147. The composition of claim **146**, wherein the single-stranded nucleic acid comprises a nucleotide sequence which is part of an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or part of an HIV-1 long terminal repeat region.

148. The composition of claim **146**, wherein the single-stranded nucleic acid comprises DNA.

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149. The composition of claim **146**, wherein the single-stranded nucleic acid comprises RNA.

150. A nucleic acid comprising a nucleotide sequence selected from the group consisting of

(i) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₂ having ATCC Accession No. 40231;

(ii) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₃ having ATCC Accession No. 40232;

(iii) a nucleotide sequence of either strand of viral DNA from clone BH10 having ATCC Accession No. 40125;

(iv) a nucleotide sequence of either strand of viral DNA from clone BH5 having ATCC Accession No. 40126;

(v) a nucleotide sequence of either strand of viral DNA from clone BH8 having ATCC Accession No. 40127;

(vi) a nucleotide sequence of either strand of viral DNA from clone pHXB3 having ATCC Accession No. 67081;

(vii) a nucleotide sequence of either strand of viral DNA from clone pHXB-2D having ATCC Accession No. 67082;

(viii) a nucleotide sequence of either strand of viral DNA from *E. coli* clone X10-1 having ATCC Accession No. 67083; and

(ix) a Human Immunodeficiency Virus Type-1 (HIV-1) nucleotide sequence from the H9/HTLV-III cell line having ATCC Accession No. CRL 8543;

wherein the nucleic acid does not form a duplex with HTLV-I and HTLV-II nucleic acids under conditions of stringency for hybridization under which the nucleic acid forms a duplex with an HIV-1 nucleic acid; and wherein the nucleic acid is covalently attached to a solid support.

151. The nucleic acid of claim **150**, wherein the nucleic acid comprises a nucleotide sequence which is part of an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or part of an HIV-1 long terminal repeat region.

152. The nucleic acid of claim **150**, wherein the nucleic acid comprises DNA.

153. The nucleic acid of claim **150**, wherein the nucleic acid comprises RNA.

154. A nucleic acid comprising a nucleotide sequence selected from the group consisting of

(i) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₂ having ATCC Accession No. 40231;

(ii) a nucleotide sequence of either strand of viral DNA from lambda bacteriophage λ -HXB₃ having ATCC Accession No. 40232;

(iii) a nucleotide sequence of either strand of viral DNA from clone BH10 having ATCC Accession No. 40125;

(iv) a nucleotide sequence of either strand of viral DNA from clone BH5 having ATCC Accession No. 40126;

(v) a nucleotide sequence of either strand of viral DNA from clone BH8 having ATCC Accession No. 40127;

(vi) a nucleotide sequence of either strand of viral DNA from clone pHXB3 having ATCC Accession No. 67081;

(vii) a nucleotide sequence of either strand of viral DNA from clone pHXB-2D having ATCC Accession No. 67082;

(viii) a nucleotide sequence of either strand of viral DNA from *E. coli* clone X10-1 having ATCC Accession No. 67083; and

(ix) a Human Immunodeficiency Virus Type-1 (HIV-1) nucleotide sequence from the H9/HTLV-III cell line having ATCC Accession No. CRL 8543;

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wherein the nucleic acid does not form a duplex with HTLV-I and HTLV-II nucleic acids under conditions of stringency for hybridization under which the nucleic acid forms a duplex with an HIV-1 nucleic acid;

wherein the nucleic acid comprises a detectable label 5
covalently attached to the nucleic acid; and
wherein the detectable label is not an additional nucleic acid.

155. The nucleic acid of claim **154**, wherein the nucleic acid comprises a nucleotide sequence which is part of an 10
HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or part of an HIV-1 long terminal repeat region.

156. The nucleic acid of claim **154**, wherein the nucleic acid comprises DNA. 15

157. The nucleic acid of claim **154**, wherein the nucleic acid comprises RNA.

158. A nucleic acid comprising a nucleotide sequence selected from the group consisting of

- (i) a nucleotide sequence of either strand of viral DNA 20
from lambda bacteriophage λ -HXB₂ having ATCC Accession No. 40231;
- (ii) a nucleotide sequence of either strand of viral DNA
from lambda bacteriophage λ -HXB₃ having ATCC
Accession No. 40232;
- (iii) a nucleotide sequence of either strand of viral DNA
from clone BH10 having ATCC Accession No. 40125;
- (iv) a nucleotide sequence of either strand of viral DNA
from clone BH5 having ATCC Accession No. 40126;
- (v) a nucleotide sequence of either strand of viral DNA 30
from clone BH8 having ATCC Accession No. 40127;

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(vi) a nucleotide sequence of either strand of viral DNA from clone pHXB3 having ATCC Accession No. 67081;

(vii) a nucleotide sequence of either strand of viral DNA from clone pHXB-2D having ATCC Accession No. 67082;

(viii) a nucleotide sequence of either strand of viral DNA from *E. coli* clone X10-1 having ATCC Accession No. 67083; and

(ix) a Human Immunodeficiency Virus Type-1 (HIV-1) nucleotide sequence from the H9/HTLV-III cell line having ATCC Accession No. CRL 8543;

wherein the nucleic acid does not form a duplex with HTLV-I and HTLV-II nucleic acids under conditions of stringency for hybridization under which the nucleic acid forms a duplex with an HIV-1 nucleic acid;

wherein the nucleic acid is outside of a mammalian cell and outside of a viral particle; and

wherein the nucleic acid is attached to a non-HIV-1 nucleic acid through a covalent bond.

159. The nucleic acid of claim **158**, wherein the nucleic acid comprises a nucleotide sequence which is part of an HIV-1 gag open reading frame, an HIV-1 pol open reading frame, an HIV-1 env open reading frame or part of an HIV-1 25
long terminal repeat region.

160. The nucleic acid of claim **158**, wherein the nucleic acid comprises DNA.

161. The nucleic acid of claim **158**, wherein the nucleic acid comprises RNA.

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